Using Phytometers to Study the Effects of Plants Diversity on Ecosystem Functioning

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Chapter 1

General Introduction

Background

Until recently, ecologists have been taking biodiversity as a function of abiotic factors. In plant communities, productivity obviously increases with soil fertility but the latter reduces biodiversity. This has sometimes been used to suggest that a decline in biodiversity might increase biotic productivity. Such simple relations are however very rare in ecology and would have contradicted for example Darwin's (1859) observation that "*If a plot of ground be sown with one species of grass, a similar plot be sown with several distinct genera of grasses, a greater number of plants and greater weight of dry herbage can be raised in the latter than the former case*." Furthermore, field observations suggested that diversity begets stability and fuller exploitation of resources because of temporal and spatial disparities in resource acquisition among species (e.g. Elton, 1958; MacArthur, 1970; McNaughton, 1993). However, it is still unclear whether biodiversity is more important as a dependent or an independent variable; and hence, what the consequences of declining biodiversity could be. Schulze and Mooney (1993) underscored the previously often ignored possibility that biodiversity may feed back on ecosystem processes. This ignited a series of experimental studies mainly with plant communities with diversity as independent and biotic productivity as dependent variable (Naeem *et al.*, 1996; Tilman, 1997; Hector *et al.*, 1999; Roscher *et al.*, 2005). The basic tenet in this work is that if biodiversity affects the means through which energy enters into an ecosystem (i.e. productivity), then it is likely that changes in biodiversity have impacts on other ecosystem processes such as nutrients cycling.

It is clear from most of the previous studies that productivity of a plant community does indeed increase with increasing diversity (synthesis by Hooper *et al.*, 2005). Following the work of MacArthur (1970) on niche differences, complementarity

in resource use is one of the mechanisms that may cause high productivity in communities that are more diverse (Hector, 1998; Hooper, 1998). This mechanism supposes that individual plants benefit from increasing diversity because of progressive replacements of conspecific neighbors by other species, thereby decreasing competition for resources. An alternative hypothesis is the increasing probability of finding a more productive species in communities with a higher number of species, commonly known as sampling effect (Aarssen, 1997; Huston, 1997). In this case, a single or few species drive biodiversity effects without other species in a community necessarily benefiting from the declining niche overlap among neighbors. Most often, the two mechanisms may occur simultaneously but with different magnitude (e.g. Hector *et al.*, 2002; Roscher *et al.*, 2005).

What did not become clear from the previous studies was how changes in plant diversity may affect a single species or a single individual plant. Positive, neutral and negative responses of individual species have been reported (Naeem *et al.*, 1996; Tilman *et al.*, 1997; Hector *et al.*, 1999; van Ruijven & Berendse, 2003; Dimitrakopoulos & Schmid, 2004). For example, in the study of Naeem *et al.* (1996), despite positive relationship between community productivity and species richness, eight of 16 species were negatively affected by increasing species richness (7 significantly) and only three of the remaining showed a significant increase. Additionally, in field studies the average individual plant biomass is often derived from the ratio of species-specific productivity to number of seeds sown (i.e. biomass per seed sown). This approach can be erroneous if plant density changes with species richness (Kennedy *et al.*, 2002; Valone & Hoffman, 2002). It is therefore necessary to assess

performance of individual plants in field conditions to confirm whether the proposed mechanism operates at the level of individual plants as supposed by the hypothesis.

High productivity, whether by complementarity or by the sampling mechanism, often corresponds to better exploitation of resources in a species-rich community. One can therefore hypothesize that any newly arriving species would find it increasingly hard to establish in communities that are more diverse. Previous support of the theory that diversity enhances invasion resistance comes from studies that use invasive species, which are often added as seeds. In this case, it is not possible to distinguish the effect of the host community (invasion resistance) and effect of the invader (invasiveness). By using native species as test invaders, one can attribute the results to invasion resistance alone.

With this background, the work in this thesis was intended to explore, whether changing plant diversity affects performance of individual plants under a given set of environmental conditions. The broader motivation was to establish the ecological consequences of changing plant diversity on individual plants. The experiments were carried out in a grassland biodiversity experiment in Jena, Germany. In one case, the performance of individuals of selected plant species that were part of the experimental communities was monitored. In another case, the performance of individuals that were transplanted into the experimental communities was monitored.

Scope of Jena Experiment

This work is part of "The Jena experiment", an integrated biodiversity project focusing on the effects of plant diversity on element cycles, multi-trophic interactions and plant

population processes (Roscher *et al.*, 2004). Overall, the experiment seeks to address some issues raised in response to findings of the first generation of biodiversity experiments (e.g. Cedar Creek, Tilman *et al.*, 2001; BIODEPTH, Hector *et al.*, 1999). The unique aspects of The Jena Experiment include nesting of diverse sub-experiments into species diversity plots, investigation of carbon storage and studies of element cycles and trophic interactions. The design is more balanced in terms of functional groups and species richness gradients than in previous experiments (Roscher *et al.*, 2004).

The experiment was established in spring 2002 on approximately 10-ha of former agricultural land in a river floodplain. The field site was divided into four blocks because of a gradient of soil characteristics, perpendicular to the river (Appendix 1). A pool of 60 grassland species that naturally occur in similar terrain of the region was used to create experimental communities (Appendix 2). The species were divided into four functional groups, namely grasses, small-herbs, tall-herbs and legumes, by an ordination procedure based on 17 functional and architectural traits. Experimental communities of monocultures, 2-, 4-, 8- and 16-species mixtures were created by random selection (with replacement) and established in plots of 20 x 20 m at a total density of 1000 viable seeds per square meter (Roscher *et al.*, 2004). In addition, four plots were seeded with all 60 species, four left bare and natural succession allowed in four plots with two of these subjected to regular mowing. In order to test specific hypotheses, 390 small-area plots (3.5 m x 3.5 m) were also set-up. Seventy-eight of these smaller plots were replicates of the main plots but with nested density and evenness treatments, 206 comprised mixtures that were created from nine potentially dominant species, and the rest were monocultures of other species. In the experiment

with dominant species, each species occurred in all diversity levels in equal frequency making it possible to assess performance of a single species across the full diversity gradient. Diversity treatments included monocultures and mixtures of 2, 3, 4, 6 and 9 species (for full description of the design see Roscher *et al.*, 2004).

Thesis Outline

Like in previous studies, community productivity was positively related to species richness in The Jena Experiment (Roscher et al., 2005). This relationship was even stronger in the experiment with dominant species where I assessed performance of individuals of four species out of the nine used in this sub-experiment. In general, both complementarity and sampling effects contributed to the overall net biodiversity effects. It was therefore probable that individual plants of the resident species benefited from increasing plant diversity. Conversely, one would expect that newly introduced species find it hard to establish in species-rich communities because the resident species already occupy most of the niche space and are using most of the resources. I transplanted four species into plots of the main experiment to address this second question.

Chapter 2 reports the effects of species richness on individuals of selected species in communities where total productivity increases with species richness. I sought to determine whether diversity benefits individual plants as predicted by niche complementarity and facilitation hypotheses.

In ***chapter 3***, I use native species to examine how diversity influences invasion in plant communities. Using native species as test invaders controlled for potential interaction between the invading species and diversity of the resident community. In

this case, the response of the test invaders can be fully attributed to diversity of the host communities.

As a follow-up of chapter 3, the experiment reported in ***chapter 4*** sought to establish the relative contribution of aboveground competition to invasion resistance. I compared the performance of individuals of one of the test invader species in full competition and with aboveground competition removed.

Lastly, in ***chapter 5*** we used the test invaders as phytometers to investigate positive interactions between particular species and legumes across a gradient of plant diversity. We assessed $\delta^{15}N$ ratios, nitrogen concentration and nitrogen content in tissues of the test invaders. Comparing the $\delta^{15}N$ ratios in transplants growing in assemblages with and without legumes should reveal nitrogen transfer from legumes, thus confirming facilitation.

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Chapter 2

Effects of diversity on growth and survival of individual plants

Manuscript

Together with M. Schmitz, M. Scherer-Lorenzen, C. Roscher, W.W. Weisser, B. Schmid

Abstract

The positive relationship between plant diversity and total community productivity suggests that individual plants are on average bigger in communities that are more diverse. However, the biomass of any species, corrected for sowing proportion, may increase with species richness both because more individuals establish or because the average individual gets bigger. We therefore studied the response of the four species *Dactylis glomerata, Phleum pratense, Geranium pratense* and *Trifolium pratense* to species richness at both the level of the entire population and the level of marked individuals. Experimental plant communities of 1, 2, 3, 4, 6 or 9 species were created from a pool of nine species. We used aboveground biomass, number of ramets, height and survival of individual plants to assess the performance of the four test species. The competitively superior grass species *Arrhenatherum elatius* negatively affected the performance of all test species except the legume *T. pratense*. Within experimental communities with or without *A. elatius*, marked individuals of *D. glomerata* responded positively, of *T. pratense* and *P. pratense* did not respond, and of *G. pratense* responded negatively to increasing species richness. Mortality of marked individuals decreased with species richness in *D. glomerata* and *T. pratense*, indicating that these two species also responded positively to species richness at the population level, producing more individuals per seed sown in species-rich plots. The two components, size and number of individuals, combined to positive population-level biomass responses of *D. glomerata*, whereas population-level responses remained neutral for *T. pratense* and *P. pratense* and negative for *G. pratense*. This reflects a transitive sequence of competitive abilities between the four test species. For the strongest competitor among them, *D. glomerata*, we could demonstrate a negative feed-back on its neighborhood in the

species-rich communities, leading to loss of correlation between local richness, local biomass, and performance of *D. glomerata*. These results demonstrate the subtlety of species-specific interactions leading to community-level biodiversity effects.

Keywords:

Biodiversity experiment, competition, individual- and population-level responses, neighborhood, species-richness effects, test plant species

Introduction

Experimental evidence shows that plant communities that are more diverse are more productive than less diverse counterparts (e.g. Tilman *et al.* 1997b; Hector *et al.* 1999; Tilman *et al.* 2001). This suggests that, on average, individuals surrounded by heterospecific neighbors outperform individuals surrounded by conspecifics; a phenomenon know as overyielding (Harper 1977; Tilman *et al.* 1996). Overyielding can occur because of complementary resource use or facilitation between species in mixtures. For example, individuals of a deep-rooting species have fewer neighbors sharing resources deep in the soil when in a mixture with a shallow-rooting species; or individuals of a non-legume species can take up nitrogen fixed by neighboring legume individuals when growing together (Vandermeer 1989). Niche complementarity may also involve differences in form and timing of resource uptake among species, allowing for chemical and temporal resource partitioning (Hooper 1998; McKane *et al.* 2002). The analysis of this phenomenon and its potential application to intercropping in agriculture has a long history in two-species mixtures (de Wit *et al.* 1966; Harper 1977; Vandermeer 1989). However, only with the recent biodiversity experiments the extension to more than two or three species has been attempted (Tilman *et al.* 1997b; Hooper 1998; Loreau & Hector 2001).

Unlike studies with two species, multi-species biodiversity experiments are more difficult to interpret because there could be many potential mechanisms leading to overyielding of the plant community as a whole. For example, in addition to complementary resource use there could be a sampling effect related to the increased chances of finding a highly productive species in species-rich communities (Aarssen 1997; Huston 1997). Furthermore, even in an overyielding mixture there could be some

species pairs with complementary or facilitative interactions, and others where one member grows at the expense of the other. To better understand the mechanisms leading to overyielding, it is therefore necessary to study the effects of species richness and more generally of plant diversity (i.e. including effects of species compositions) on the performance of individual species and even individual plants (Naeem *et al.* 1996; Dimitrakopoulos & Schmid 2004). In previous experiments, which have done the first of these two things, positive responses to increasing species richness have been found for some species but not others (Naeem *et al.* 1996; Tilman *et al.* 1997a; Hector *et al.* 1999; van Ruijven & Berendse 2003; Dimitrakopoulos & Schmid 2004). However, because in these analyses the species responses were expressed per individual sown or planted, they reflected population-level responses: a positive response to species richness could have occurred because more individuals established or because the established individuals were bigger in species-rich than in species-poor communities. In some cases, diversity has indeed been shown to enhance total community density (Kennedy *et al.* 2002) or population size of individual species (Valone & Hoffman 2002).

In the current study, we analyzed the response to increasing species richness in four test species at both the population and the individual level. We measured the performance of marked individuals of these species and of the surrounding community in experimental grassland plots established from a pool of nine potentially dominant species. Following an old quest (Harper 1967), our goal was to establish the missing links between the aggregated measures of community and species productivity and the performance of individual plants in biodiversity experiments, at least for four species representing the three functional groups grasses, legumes and tall herbs (Roscher *et al.*

2004). Specifically we addressed the following questions: (a) is increasing community productivity with increasing species richness paralleled by increasing average size of individuals of the different species; (b) is increasing community productivity with increasing species richness paralleled by increasing community density, i.e. increasing establishment success of the different species; (c) how does the presence or absence of particular species in experimental plots affect the responses of the test species at population and individual level?

Material and methods

Experimental site and design

This study was carried out on plots of a large-scale grassland biodiversity experiment in Jena, Germany ("The Jena Experiment", http://www.the-jena-experiment.de). The experiment was set up in spring 2002 on former agricultural land in the flood plain of the river Saale (Roscher *et al.* 2004). The site was divided into four blocks following a gradient in soil characteristics perpendicular to the river Saale. Experimental communities were established from a pool of nine potentially dominant species, which nevertheless varied considerably in monoculture yields. They included five grasses, *Alopeculus pratense* L., *Arrhenatherum elatius* L., *Dactylis glomerata* L., *Phleum pratense* L. and *Poa trivialis* L., two non-legume herbs, *Anthriscus sylvestris* L. and *Geranium pratense* L., and two legumes, *Trifolium pratense* L. and *Trifolium repens* L. Monocultures and 2-, 3-, 4-, 6- or 9-species mixtures were created, with each species occurring in eight different mixtures per richness level > 1. Each monoculture and each mixture was replicated in two plots (Table 2.1). It was therefore possible to evaluate the

performance of a single species across the full species richness gradient (n = 74 plots per species). The plots measuring 3.5 × 3.5 m were seeded at a total density of 1000 viable seeds per square meter. We maintained the initial species richness in the plots by removing unsown plants in April and July each year and the plots were mown at the end of May and August each year.

Table 2.1 Number of plots for each of four test species and total number of plots at each species richness level in the experiment (plots with more than one test species could be used in more than one separate analysis of test species; see also Fig. 2.3).

Species richness	Species composition (= mixture term)	Number of different mixtures per test species	Number of plots per test species	Total number of plots per richness level
1	Monoculture, i	1	2	18
2	i + 1 other species	8	16	72
3	i + 2 other species	8	16	48
4	i + 3 other species	8	16	36
6	i + 5 other species	8	16	24
9	i + 8 other species	1	2[1]	8
		34	68	206

[1] Each test species was marked only in 2 of the 8 highest-richness plots. Thus, at this richness level, plots were never used for more than one target species at the same time.

Target species and data collection

We chose four of the nine species, *D. glomerata, P. pratense, T. pratense, G. pratense*, as our target species. We avoided *A. elatius* because of its high competitive dominance, *A. pratense, P. trivialis* and *T. repens* because of their stoloniferous growth form, which would have made it hard to distinguish individuals, and *A. sylvestris* because of its initial poor establishment. Between 23 and 30 July 2002, 2 months after sowing, ten

individuals of each target species were marked in each experimental plot where they occurred. For each species, individuals nearest to 30-cm interval marks along a line 30 cm parallel to one edge of the plot were tagged with a white plastic label fixed next to the plant. Each label was numbered to ease identification during data collection. Fewer than ten individuals per plot were marked in *G. pratense*, which had poorly germinated. Before mowing at the end of August 2002, we measured the height and counted the number of ramets (number of leaves in *G. pratense*) of all marked individuals. Then we cut them at 3 cm from the ground and determined the dry mass of the aboveground plant parts. Except for *G. pratense*, where not enough individuals re-established, the marked plants were censured early in spring 2003 and after every succeeding mowing in 2003 and 2004 to monitor their mortality, without further harvesting.

Between 26 May and 4 June of the following year, 2003, we marked five new plants of each target species. We again recorded their height and number of ramets at the peak of each growing season and cut the plants at 3 cm above the ground to determine their average aboveground biomass, pooling individuals of the same species per plot.

Species-specific and community measurements

During each growing season, community and species-specific aboveground biomass was determined in a randomly selected 20 x 50 cm (0.1 m^2) area in each of the 206 plots. In the present study, we are using the biomass harvests from spring 2003 and spring 2004 to assess biodiversity effects on population and community levels. For the analysis, species-specific biomass values were multiplied by species richness to correct for the decreasing proportion with increasing species richness and to make the values

qualitatively comparable with whole-plot community biomass. We refer to this corrected biomass as a population-level variable. We also recoded the canopy height before each harvest in August 2002, 2003 and 2004 and in May 2004. In addition, we estimated the total density of plants by counting the number of rooted individuals in an area of 10 x 100 cm after the spring mowing in 2003.

Dactylis glomerata neighborhood

Between 24 May and 4 June 2004, for each plant of *D. glomerata* marked in spring 2003, we in addition counted the number of ramets of each neighboring species within a radius of 12.5 cm to determine neighborhood density and diversity. Comparable studies suggest that this neighborhood size is adequate for neighborhood analysis (e.g. Kennedy *et al.* 2002). Subsequently, we harvested the aboveground biomass of this neighborhood at 3 cm above the ground (harvested area per plot = $0.245 m^2$), separated the material into species and determined their biomass.

Statistical analysis

We analyzed plot means of the variables measured on the marked individuals of the test species and of the population and the community variables with general linear models using sequential sums of squares (Schmid *et al.* 2002). The mortality rate of the marked individuals was analyzed with a generalized linear model, using binomial errors and a complementary log-log link (Egli & Schmid 2001). We used the number of plants that died in each time interval as the response variable. Biomass data were log-transformed before analysis to improve the normality of residuals.

Our initial statistical model included block, sown species richness, species composition (= mixture), plot and census in a repeated-measures approach. However, when we found a strong contrast for the presence or absence of the highly competitive *A. elatius* in mixtures (explaining most of the species composition = mixture effects), we removed the effect of this species before analyzing effects of species richness in subsequent analyses. Because there was no interaction between species richness and census, we only report mortality results for the July 2002 cohort. The hierarchical nature of our design required that we use several error terms for testing the significance of terms: block and mixture were tested against plot, presence of *A. elatius* and species richness against mixture and the remaining terms were tested against the residuals. We partitioned species richness into either a log-linear or a linear contrast and corresponding deviations, as is usually done in the analysis of biodiversity experiments (Schmid *et al.* 2002). We then selected the contrast that had a higher sum of squares for the particular variable; if the deviation was small, we pooled it with the mixture term. Most interactions had P values > 0.1; hence, they are not reported here. All analyses were calculated with the software product Genstat 6th Edition, Release 6.2 (Payne *et al.* 2002).

Results

Effects of diversity on the entire community and on the populations of the test species

In the community-level analysis of all 206 plots, peak community aboveground biomass increased linearly with the logarithm of species richness (Table 2.2, Fig. 2.1a). This was paralleled by an increase in canopy height (log-species richness: $F_{1,94}$ = 27.91, P <

0.001). Communities in which *A. elatius* was present on average had a higher biomass than plots without this grass species (Table 2.2, Fig. 2.1a). Only at high richness levels (4 or 6 species) the presence of *A. elatius* was no longer "necessary" to reach high yield, which was reflected in a significant interaction of the presence of *A. elatius* and the logarithm of species richness (Table 2.2, Fig. 2.1a). The community biomass also varied considerably among different species composition and between the two years, and the effect of species richness was weaker in 2004 than in 2003 (Table 2.2).

Table 2.2 Summary analyses of variance of spring 2003 and spring 2004 aboveground biomass of the test species (population-level, n = 68) and the entire community (n = 206) showing F and P values. Population-level data were obtained by multiplying component yields of species with species richness to correct for decreasing sowing proportions with increasing richness level. Log-SR is the logarithm of species richness.

		D. glomerata		*P. pratense*		*T. pratense*		*G. pratense*		Community	
Source of variation	d.f.	F	P	F	P	F	P	F	P	F	P
Block	3	8.24	**<0.001**	1.15	0.341	3.38	**0.028**	3.38	**0.029**	9.71	**<0.001**
Presence of *A. elatius*	1	59.93	**<0.001**	15.81	**<0.001**	7.02	**0.013**	10.31	**0.003**	54.11	**<0.001**
Log-SR	1	32.86	**<0.001**	2.32	0.138	0.59	0.447	8.91	**0.006**	23.89	**<0.001**
Pres. *A. e.* x Log-SR	1	4.08	0.052	0.02	0.899	0.01	0.940	2.74	0.109	12.41	**0.001**
Mixture	31	0.70	0.847	5.15	**<0.001**	1.04	0.448	2.82	**0.002**	2.10	**<0.001**
Plot	36	1.72	**0.026**	0.73	0.849	1.68	**0.031**	0.91	0.605	0.76	0.944
Year	1	15.45	**<0.001**	68.24	**<0.001**	18.43	**<0.001**	2.91	0.093	120.72	**<0.001**
Log-SR x Year	1	0.21	0.648	3.11	0.082	3.78	0.056	3.41	0.070	18.23	**<0.001**

The population-level analyses showed that, species richness had a positive effect on biomass (corrected for sowing proportion) in *D. glomerata*, no significant effect in *P. pratense* and *T. pratense* and a negative effect in *G. pratense* (Table 2.2, Fig. 2.1b-e). The presence of *A. elatius* in mixtures reduced the biomass of all four target species while further effects of particular species compositions were relatively small (Table 2.2).

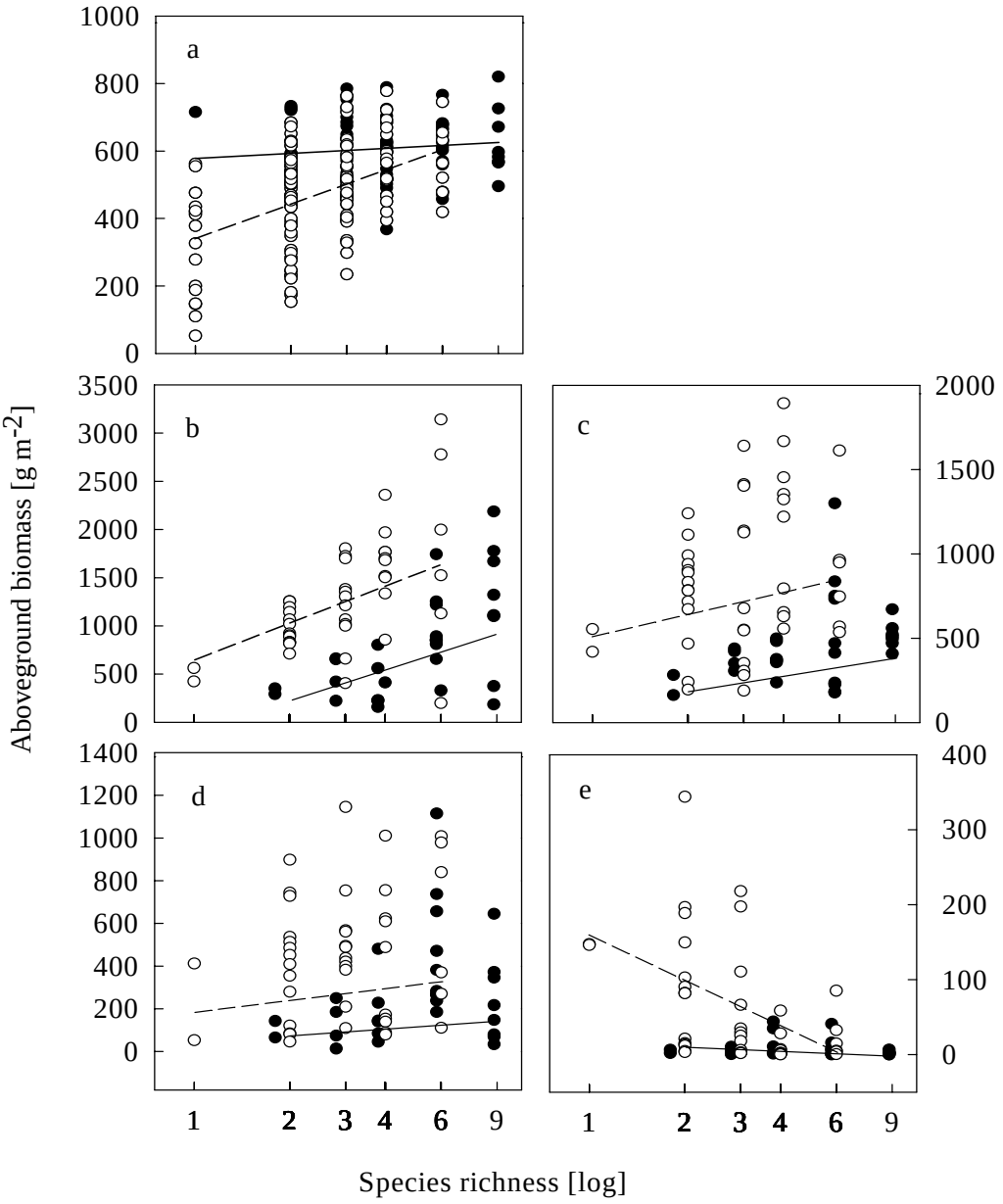

Figure 2.1 Average (spring 2003 and spring 2004) aboveground biomass of the entire community (a; n = 206) and of populations of the test species (a) *D. glomerata*, (b) *P. pratense*, (c) *T. pratense*, (d) *G. pretense* (all n = 68) as a function of the logarithm of species richness. The observed values for the species were multiplied by the corresponding richness level to correct for decreasing sowing proportions with increasing richness. Plots with *A. elatius* are shown with filled circles and continuous lines; plots without *A. elatius* with open circles and broken lines.

Total community density of plants in spring 2003 (n = 206) was slightly higher in plots with *A. elatius* ($F_{1,96}$ = 3.81, P = 0.054) and tended to increase with the

logarithm of species richness ($F_{1,96}$ = 3.67, P = 0.058). The low significances for these trends were due to the large and significant variation in the corresponding error term, i.e. differences between particular species compositions within richness levels ($F_{96,103}$ = 2.60, P < 0.001). Community density ranged from less than 100 plants / m^2 in mixtures without grasses to over 500 plants / m^2 in mixtures containing only grasses. As a covariate, community density did not explain a significant amount of variation in community biomass nor in the biomass of any of the test species.

The presumed gradient in soil characteristics (block effect) had significant effect on the community and population biomass of the test species except *P. pratense*, indicating the importance of environmental heterogeneity. Plants biomass decreased with distance from the river.

Effect of diversity on individuals of the test species

When the competitively strong grass *A. elatius* was present in a mixture, it strongly reduced the biomass of marked individuals of all test species except *T. pratense* (Table 2.3, Fig. 2.2). The size reduction was 41% in *D. glomerata,* 20% in *P. pratense* and 53% in G. *pratense*. Similarly, the presence of *A. elatius* in mixtures had negative effects on the number of shoots (leaves in *G. pratense*) and the height of marked individuals of all target species except *T. pratense*. In *D. glomerata*, the number of shoots declined by 33% ($F_{1,30}$ = 21.57, P < 0.001) and the height by 9% ($F_{1,30}$ = 7.88, P = 0.009), in *P. pratense* the number of shoots declined by 5.4% ($F_{1,30}$ = 16.57, P < 0.001) and the height 4% ($F_{1,30}$ = 4.42, P = 0.044) and in *G. pratense* the number of leaves declined by 26% ($F_{1,30}$ = 7.03, P = 0.013).

Table 2.3. Analyses of variance of the average aboveground biomass of marked individuals of the four test species. Log-SR is the logarithm of species richness. Interaction terms with F < 2 are not shown, although they were included in the statistical model.

	D. glomerata				*P. pratense*				*T. pratense*				*G. pratense*			
Source of variation	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P
Block	3	3.76	4.79	**0.007**	3	3.56	5.15	**0.005**	3	6.35	4.12	**0.015**	3	6.47	2.13	0.117
Presence of *A. elatius*	1	28.98	29.69	**<0.001**	1	59.42	26.28	**<0.001**	1	1.58	0.88	0.357	1	109.56	22.18	**<0.001**
Log-SR	1	5.31	5.44	**0.027**	1	4.61	2.04	0.164	1	1.00	0.55	0.463	1	84.02	17.01	**<0.001**
Pres. *A. e.* x Log-SR	1	0.13	0.13	0.721	1	0.07	0.03	0.860	1	7.66	4.24	**0.048**	1	18.49	3.74	0.063
Mixture	30	0.98	1.24	0.274	30	2.26	3.27	**0.001**	30	1.81	1.17	0.334	30	4.94	1.63	0.094
Plot	31	0.78	4.96	**<0.001**	31	0.69	2.53	**<0.001**	30	1.54	1.52	0.076	30	3.03	7.65	**<0.001**
Harvest	3	36.20	228.99	**<0.001**	3	93.61	342.21	**<0.001**	2	79.65	78.28	**<0.001**	1	2.46	6.21	**0.016**
Log-SR x Harvest	3	0.20	1.26	0.291	3	0.27	0.97	0.408	2	0.46	0.45	0.639	1	0.24	0.60	0.444
Residual	197	0.16			194	0.27			74	1.02			54	0.40		
Total	270	0.89			267	1.87			144	2.54			122	4.06		

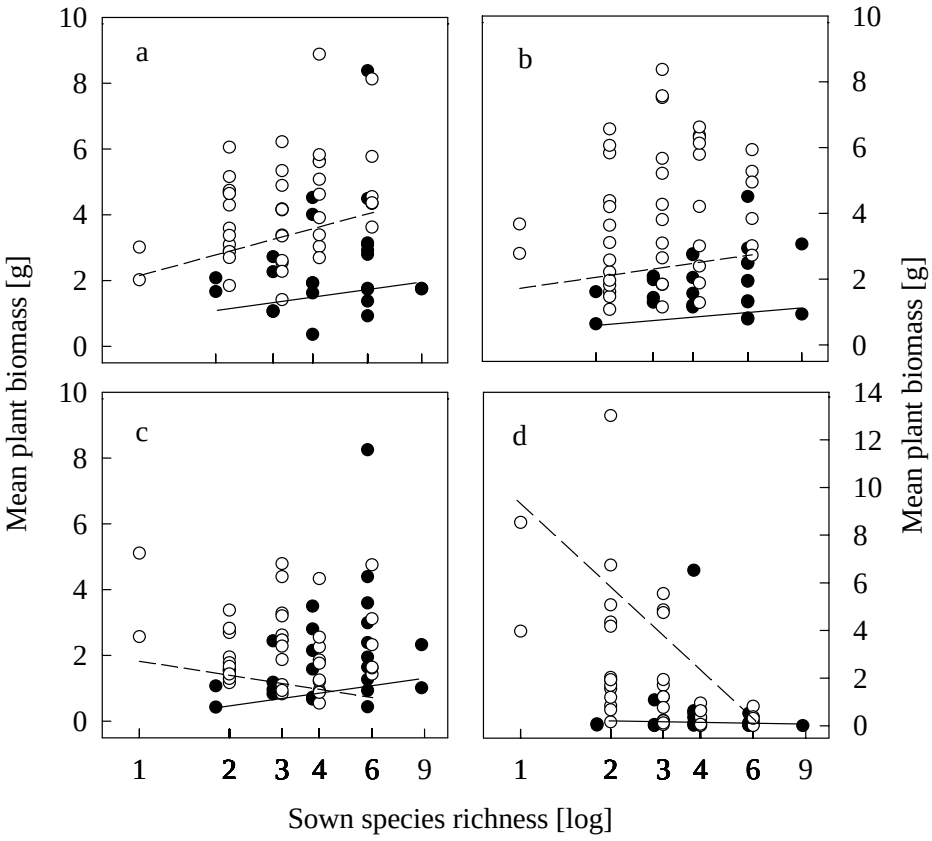

Figure 2.2 Average (2003 and 2004) aboveground biomass of marked individuals of the test species (a) *D. glomerata* (four harvests), (b) *P. pratense* (four harvests), (c) *T. pratense* (three harvests) and (d) *G. pratense* (three harvests) as a function of the logarithm of species richness. Plots with *A. elatius* are shown with filled circles and continuous lines; plots without *A. elatius* with open circles and broken lines. Note that the points are staggered along the x-axis for clarity and that few of the marked individuals of *G. pratense* survived in plots with *A. elatius*.

After factoring out the effects of *A. elatius*, species richness had positive effects on individual biomass in *D. glomerata* and negative effects in *G. pratense* (Table 2.3, Fig. 2.2). In addition, the negative effect of species richness on *G. pratense* was stronger in the absence than in the presence of *A. elatius* but not statistically significant because of high mortality of this test species in communities with in *A. elatius* (Table 2.3, Fig. 2.2). The biomass of marked individuals of the other two test species was not influenced by species richness, which corresponds to their non-significant responses to species richness at the population-level (see above). There was, however, a significant

interaction effect of species richness and presence of *A. elatius* on biomass of *T. pratense* (Table 2.3, Fig. 2.2).

Species richness had no effect on the number of modules (shoots) on the marked individuals of the test species except *G. pratense* where number of leaves declined with increasing species richness. However, individuals of *P. pratense* were taller in species-rich mixtures ($F_{1,30}$ = 9.08, P = 0.005), whereas those of *G. pratense* were taller in species-poor mixtures ($F_{1,30}$ = 8.46, P = 0.007). Furthermore, species composition had a strong influence on biomass of marked individuals of *P. pratense* (mixture term in Table 2.3) and height and number of modules in marked individuals of *P. pratense* (shoots) and *G. pratense* (leaves).

Rates of mortality of marked individuals within the first year were 82%, 49% and 40% for *T. pratense*, *D. glomerata* and *P. pratense*, respectively, and then declined considerably in the second year (first year contrast and remaining time intervals in Table 2.4, Fig. 2.3). The presence of *A. elatius* in mixtures increased the risk of mortality of the two grass test species (Table 2.4) but not of *T. pratense*, perhaps due to a legume-grass complementarity in the latter. Increasing species richness (in this case using the linear rather than the log-linear contrast) reduced the risk of mortality of *D. glomerata* and *T. pratense* individuals after taking into account the effect of *A. elatius.* Furthermore, mortality risk in *P. pratense* and *T. pratense*, but not in *D. glomerata*, was significantly affected by particular species compositions. For example, mortality of *P. pratense* individuals was particularly high in mixtures containing *Poa trivialis*.

As in the community- and population-level variables, the presumed gradient in soil characteristics (block effect) had a significant effect on the performance and

mortality of marked individuals (Table 2.3 & 2.4), indicating the importance of environmental heterogeneity on plant performance.

Table 2.4 Analyses of deviance of the mortality risk of marked individuals of three of the four test species. The response variable was the number of plants that died during a time interval out of those at the beginning of the interval. The block term was tested against the plot error, while diversity terms above mixture were tested against mixture as error term (see "Material and methods"). M.Dev. is the mean deviance change and F is the deviance ratio for the quasi-F test with * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.

		D. glomerata		*P. pratense*		*T. pratense*	
Source of variation	d.f.	M.Dev.	F	M.Dev.	F	M.Dev.	F
Block	3	72.10	**23.23*****	22.94	**17.13*****	7.4	**6.31****
Biomass in August 2002	1	2.45	1.12	5.27	2.09	8.27	2.12
Presence of *A. elatius*	1	9.84	**4.52***	26.32	**10.41****	0.58	0.15
Sown species richness (SR)	1	10.33	**4.74***	0.03	0.01	16.02	**4.12***
Mixture	31	2.18	0.70	2.53	**1.89***	3.89	**3.30*****
Plot	30	3.10	**3.06*****	1.34	1.01	1.18	0.56
First year contrast	1	103.58	**102.25*****	2.63	1.98	6.09	2.90
Remaining time intervals (T)	3	9.22	**9.10*****	16.23	**12.18*****	34.65	**16.50*****
SR x first year contrast	1	0.15	0.15	<0.01	<0.01	1.90	0.91
SR x T	3	0.86	0.85	0.74	0.56	1.48	0.71
Mixture x T	125	1.45	**1.43***	1.30	0.98	1.41	0.67
Residual	126	1.01		1.33		2.10	

For *P. pratense* and *T. pratense*, d.f. are: Plot 29 and 29, Mixture x T 128 and 107, Residual 128 and 73.

Effect of local neighborhood on individuals of Dactylis glomerata

In spring 2004, the aboveground biomass and number of shoots of the population of *D. glomerata* within a 12.5-cm radius neighborhood around marked individuals of this species increased with linearly increasing species richness, if corrected for the decreasing sowing proportion (biomass: $F_{1,30} = 5.3$, P = 0.028, Fig. 2.4a; number of shoots: $F_{1,30} = 21.54$, P < 0.001, Fig. 2.4b), again indicating overyielding of this test species at the population level (Fig. 2.5). At the community level, the total aboveground biomass and number of shoots of all species in the neighborhood around the marked individuals of *D. glomerata* was not affected by linear species richness nor by species

composition, although the number of species in this local area correlated well with sown species richness ($r^2 = 0.79$, $F_{1,28} = 118.9$, $P < 0.001$).

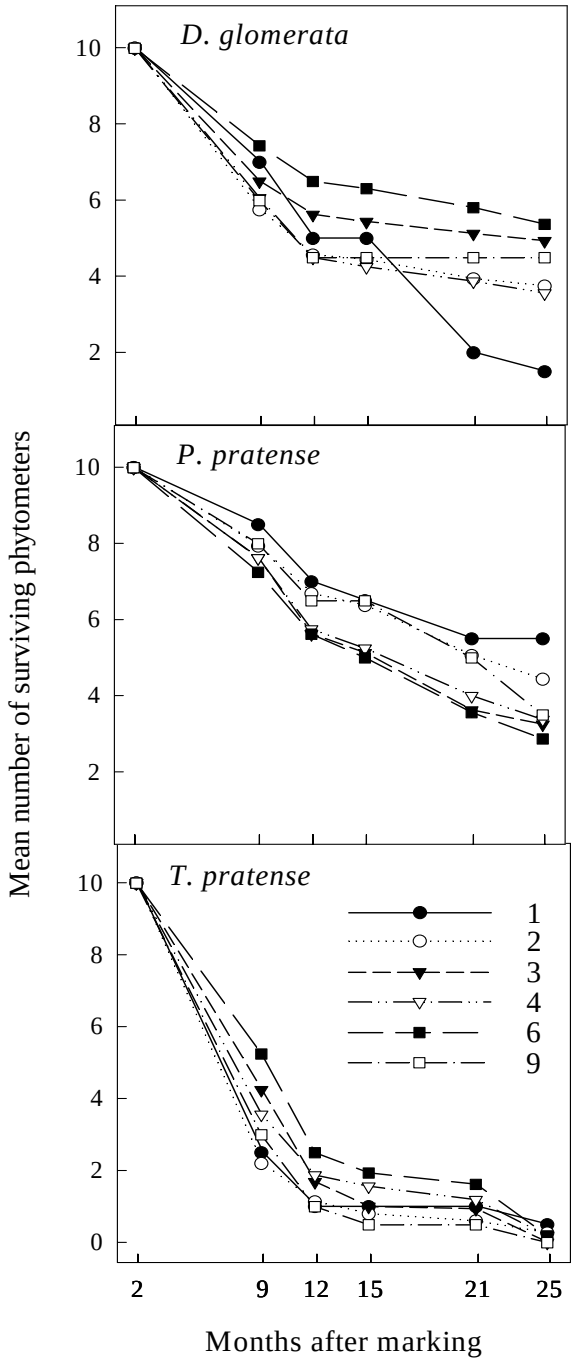

Figure 2.3 Average survivorship curves of ten individual per plot per test species marked in July 2002 and monitored at different time intervals until August 2004. Note since too few individuals of the forth test species, *G. pratense* were there by the second census this species was not included in this analysis.

As covariates, these neighborhood attributes (community-level) had no effect on the performance of marked individuals of *D. glomerata* and did not change the pattern of effects of diversity treatments on the biomass of these individuals, i.e. effects of the presence or absence of *A. elatius* ($F_{1,31}$ = 55.92, P < 0.001) and species richness (linear contrast $F_{1,31}$ = 4.7, P = 0.038) remained significant (Fig. 2.4c). The number of shoots per marked individual of *D. glomerata* was also still negatively affected by presence of *A. elatius* ($F_{1,31}$ = 20.0, P < 0.001) after correcting for neighborhood attributes but not affected by species richness (linear contrast $F_{1,31}$ = 1.53, P > 0.1) (Fig. 2.4d).

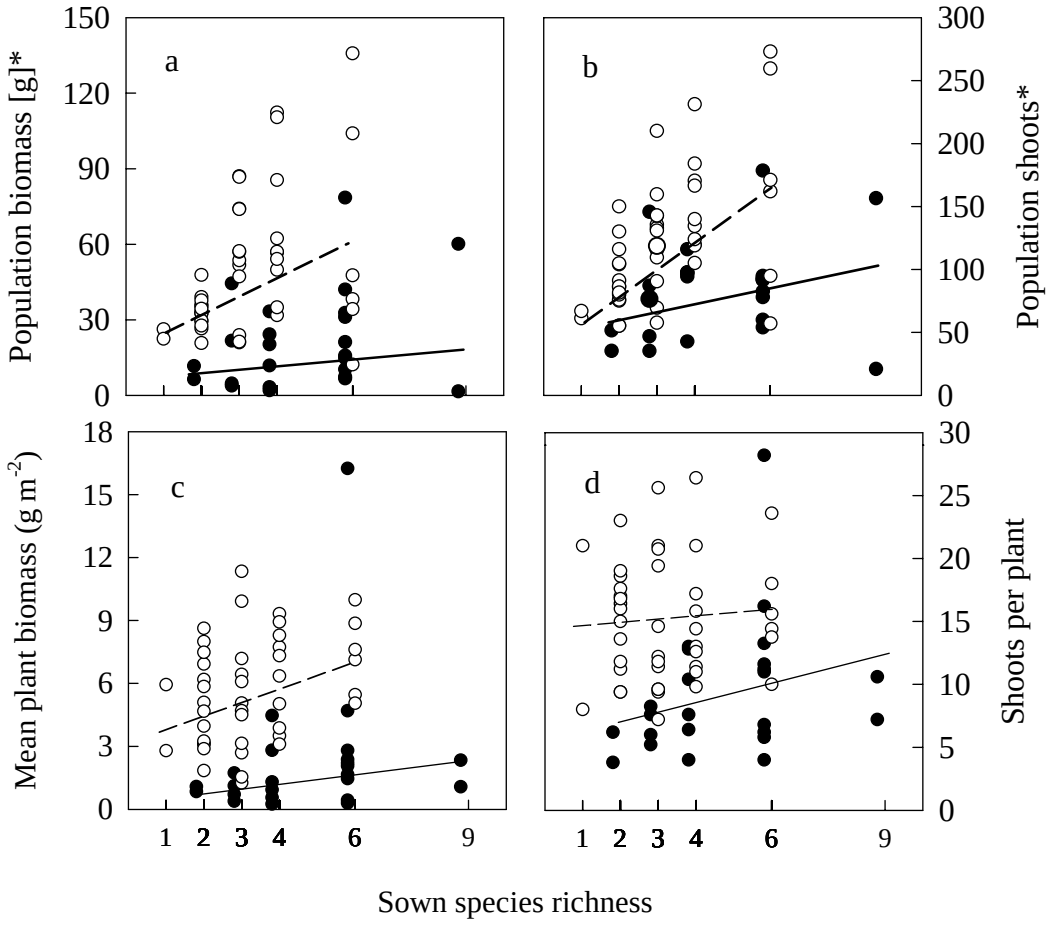

Figure 2.4 Comparison of effects of sown species richness on the individuals-level performance measures (c) plant biomass and (d) number of shoots of marked individuals of *D. glomerata* and the population-level performance measures (a) biomass and (b) number of shoots of *D. glomerata* within a 12.5-cm radius neighborhood (*) of the marked individuals in spring 2004. Plots with *A. elatius* are shown with filled circles and continuous lines; plots without *A. elatius* with open circles and broken lines.

Discussion

Effects of diversity on the entire community and on populations of the test species

As in many other biodiversity experiments (Hooper *et al.* 2005), a positive relationship between species richness and community biomass has been found in the experiment at Jena (Germany) using a pool of nine dominant species (Roscher *et al.* 2005). In our present study, we could test if this community-level response was paralleled by population- and individual-level responses in four members of the species pool. In the previous biodiversity experiments population-level responses varied between species, with some species contributing more than others to the community-level response and some species even responding negatively to increasing species richness (Naeem *et al.* 1996; Tilman *et al.* 1997a; Hector *et al.* 1999; van Ruijven & Berendse 2003). We found all these types of responses among our four test species: the grass *D. glomerata* increased its biomass per seed sown with increasing species richness, the grass *P. pratense* and the legume *T. pratense* did not respond significantly and the herb *G. pratense* showed a decreasing response. These differences can be interpreted to be a consequence of decreasing competitiveness between the four test species and the other species in the pool.

The most competitive species in the pool, the grass *A. elatius*, which was not included as test species, contributed so strongly to community biomass that we had to analyze the species richness effects within two groups of communities, with or without *A. elatius*. The presence of *A. elatius* in mixtures had a negative effect on the two grass test species and on *G. pratense,* but not on the legume *T. pratense*. Again, this result can be interpreted by competitive inferiority of three of the test species relative to the most competitive species of the pool. Presumably, the legume species could avoid

competitive suppression by this grass species due to its capability to fix atmospheric nitrogen, while the grasses themselves could not do so (Fargione *et al.* 2003).

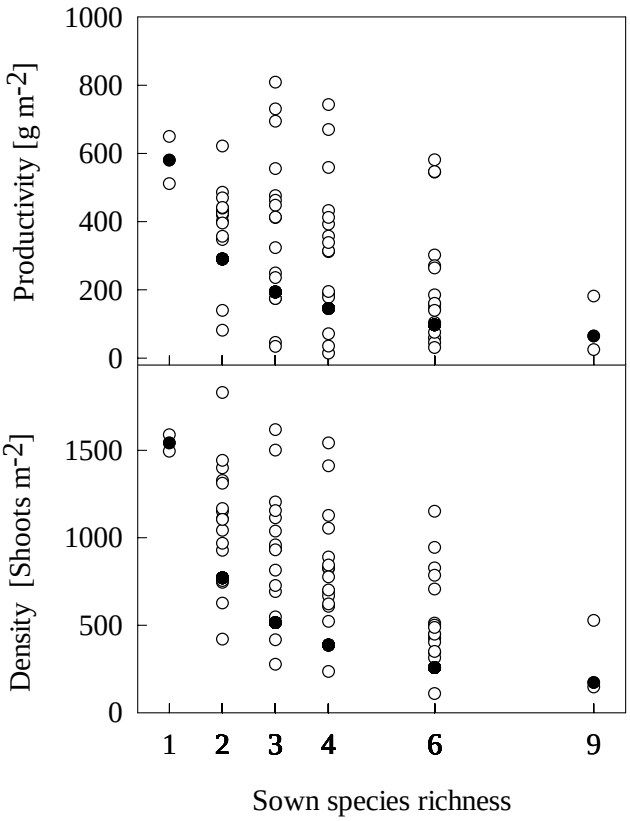

Figure 2.5 Effects of species richness on aboveground biomass (upper panel) and density of shoots (lower panel) of *D. glomerata* populations in local neighborhoods of marked individuals of this species in spring 2004 (excluding marked individual and not corrected for decreasing sowing proportions with increasing sown species richness). Filled symbols: expected yield value (i.e. yield in monoculture divided the number of species in the mixture), open symbols: observed yield values. Open symbols above the line connecting expected yield values indicate overyielding.

The weak competitive ability of the herb *G. pratense* both in relation to the presence of *A. elatius* and to increasing species richness in mixtures might have been due to its late germination. Watkinson (1997) asserts that plants germinating even ten days late may achieve negligible biomass in competition with neighbors. Fridley (2003) took into account differences in time of germination in the analysis of his biodiversity experiment. In the present study, we could not do this. Instead, we marked individual plants of *G. pratense* and the other test species to follow more closely their fate as a population in the different experimental communities.

Effects of sown diversity on individuals of the target species

The marking of individuals allowed us to investigate if the population-level responses of the four test species were due to differential establishment after sowing, i.e. different numbers of individuals in the populations, or to differential growth of individuals. Previous studies had to assume that establishment probability did not change between species richness levels to infer individual-level responses. While this may be the case if individual seedlings are planted and mortality is zero (e.g. Dimitrakopoulos & Schmid 2004), it may be less likely in biodiversity experiments which are set up by sowing seeds. Indeed, there are indications that community density of plants can increase with sown species richness (Kennedy *et al.* 2002; Schmitz *et al*., unpublished data).

Marked individuals of two of our test species, *D. glomerata* and *T. pratense*, did have a decreased mortality risk in species-rich communities; and this should have led to increased numbers of established individuals per seed sown. However, we could not find a significant decrease in mortality risk in the other two test species (for *G. pratense* there were not enough individuals for a statistical test). This suggests that the first two species might have responded more strongly to their own sowing density, which decreased with increasing species richness, whereas the other two responded more strongly to the overall sowing density, which remained constant across richness levels. Alternatively, *D. glomerata* and *T. pratense* individuals might have perceived low effective density in mixtures while the other two perceived higher effective density in mixtures despite constant sowing density (Connolly, 1986). The differential mortality responses between species resulted in a slight increase of total community density (established individuals) with increasing species richness. It is well known that mortality and individual size are density-dependent attributes in mono-specific plant

stands (Watkinson 1997); however, it is less clear how these effects operate in mixed stands.

With regard to response of the marked individuals to increasing species richness (and thus decreasing within-species density), plant biomass in *D. glomerata* increased and subsequently the population-level biomass, plant height in *P. pratense* increased but there was no response in *T. pratense*. The latter two species also showed no response at the population-level. On the other hand, plant biomass, height and number of leaves in *G. pratense* decreased, corresponding to population-level response. Thus, it appears that in all our test species population responses were more or less paralleled by individual responses. Nevertheless, at the same time there was also a "pure" population-level component, reflected in the decreased mortality risk with increasing species richness for *D. glomerata*. The effects of species richness and that of the presence of the strongest competitor, *A. elatius*, in our experimental communities seemed to be absorbed by the test plants in a balanced way: they adjusted numbers and sizes of individuals within their populations in a parallel manner.

Again, the different responses of the four test species at the individual level can be interpreted by a transitive ranking in competitive ability in the order *D. glomerata* > *T. pratense* and *P. pratense* > *G. pratense*. It then follows that for *D. glomerata* an increase in the frequency of interspecific neighbors with increasing species richness reduced competition for marked individuals, whereas for *T. pratense* and *P. pratense* inter- and intraspecific neighbors had about equal effects and for marked individuals of *G. pratense* interspecific neighbors were more detrimental than intraspecific neighbors were. Consequently, and as demonstrated in a competition experiment by Stoll & Prati (2001), weak competitors such as *G. pratense* may best establish and grow in

monospecific patches and therefore benefit from aggregated species distributions, whereas the opposite should be the case for strong competitors such as *D. glomerata* and *A. elatius*. These species should do best in regular mixtures, and indeed the latter seems to benefit strongly when grown in species-rich mixtures (Roscher *et al.*, unpublished data).

Effects of local neighborhood on individuals of Dactylis glomerata

To test the effects of local neighborhood rather than plot-wide, "global" neighborhood at least in the one test species that benefited most from a replacement of intraspecific by interspecific neighbors, we determined the richness, the number and the biomass of neighbors within circles of 12.5 cm radius around marked individuals of *D. glomerata*. Neither local species richness nor local community density around the marked individuals correlated significantly with their biomass, whereas increasing sown (global) richness not only increased the biomass of the marked individuals (see above) but also the biomass and number of shoots of the *D. glomerata* population and the biomass of the other *D. glomerata* individuals in the neighborhood of the marked individuals (see Fig. 2.4). Furthermore, there was no significant "local" relationship between community biomass and realized species richness in the neighborhood of marked individuals of *D. glomerata*. This indicates that the marked and neighboring individuals of this species had themselves had an influence on their neighborhood community: the performance of *D. glomerata* was high in plot with high sown (global) richness. This might have had a negative effect on other species in the neighborhood reducing thus the neighborhood biomass (i.e. excluding biomass of the marked

individual) and therefore depressing the effect of realized species on the neighborhood community biomass.

Conclusions

Our results show a good correlation between performance of individual plants and their respective populations. This supports previous studies that inferred performance of individual plants from species-specific biomass (e.g. Tilman *et al.* 1997a; Hector *et al.* 1999). In addition, our results show that population biomass is not only due to increased individual size but also to increased numbers of individuals per seed sown, i.e. to demographic processes. Both the growth of individuals and population size are regulated and limited by availability of resources (Watkinson 1997). Diversity effects on resources available to individuals also benefit the species populations as a whole and subsequently the entire community.

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Chapter 3

Niche pre-emption increases with species richness in experimental plant communities

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Abstract

In plant communities, invasion resistance may increase with diversity because empty niche space decreases simultaneously. However, it is not clear if this only applies to exotic species or also to native species arriving at a site with few other native species during community assembly. We tested the latter by transplanting four native species into experimental grassland communities varying in species richness form 1–16 (–60) species. In addition, we tested the hypothesis that invasion is less successful if the invading species belongs to a functional group that is already present in the community. The test invaders included a grass species (*Festuca pratensis,* FP), a short (*Plantago lanceolata*, PL) and a tall herb species (*Knautia arvensis,* KA), and a legume species (*Trifolium pratense,* TP). The same four functional groups also occurred alone or in all possible combinations in the different experimental communities. The overall performance of the transplants was negatively related to the logarithm of the species richness of host communities. Plant biomass declined by 58%, 90%, 84% and 62% in FP, PL, KA and TP, respectively, from monocultures to 16-species mixtures, indicating lower invasiveness of the two herbs than of the grass and the legume. Resident grasses showed a strong negative effect on the performance of all test invaders, whereas resident small and tall herbs had neutral, and resident legumes had positive effects. The case of the legumes indicates that contributions to invasion resistance need not parallel invasiveness. Communities containing resident species of only one functional group were most inhibitive to transplants of the same functional group. These results indicate that invasion resistance of experimental plant communities is related to the degree of niche overlap between resident species and invaders. This niche overlap can be high due to generally low amounts of empty niche space in species-rich resident communities or

due to the occurrence of the same functional group as the one of the invader in the resident community. Stronger within- than between-functional-group invasion resistance may be the key mechanism underlying diversity effects on invasion resistance in grassland and other ecosystems at large.

Key words:

diversity effects; invasion resistance; invasiveness; niche overlap; phytometers; plant functional groups; The Jena Experiment

Introduction

Understanding the mechanisms behind the relationship between resident species richness and the establishment of non-resident species (i.e. invaders in the broad sense) in natural communities is a major goal in ecology. This relationship has potential applications in conservation, restoration and prediction of community invasion resistance. Elton (1958) and Levine & D'Antonio (1999) provide evidence for a generally negative relationship between diversity and the likelihood that an intruder will be able to establish itself in a community. Such relationships have been found in a large number of experimental studies using temperate plant communities (Tilman, 1997; Knops *et al.*, 1999; Joshi *et al.*, 2000; Naeem *et al.*, 2000; Prieur-Richard *et al.*, 2000; Diemer & Schmid, 2001; Kennedy *et al.*, 2002; Pfisterer *et al.*, 2004). In contrast, observational studies, which necessarily assess invasion by exotic invaders, often report higher numbers of invading species in species-rich than in species-poor plant communities (Stohlgren *et al.*, 1999; Stadler *et al.*, 2000; Pysek *et al.*, 2002; Stohlgren *et al.*, 2002), though Stohlgren *et al.* (1999) found the opposite at one site in their study. The results of observational studies may be attributed to uncontrolled extrinsic factors, whose effect on native and exotic species is the same (Stohlgren *et al.*, 1999; Levine, 2000; Shea & Chesson, 2002). Additionally, observational studies mostly analyse the number of invading species (e.g. Stohlgren *et al.*, 1999; Meiners *et al.*, 2004) whereas many experimental studies also assess the performance of particular invaders (see e.g. Prieur-Richard *et al.*, 2000; Diemer & Schmid, 2001; Hector *et al.*, 2001).

In most cases, species richness is the only component of diversity manipulated in experimental studies (e.g. Prieur-Richard *et al.*, 2000; Troumbis *et al.*, 2002), though some studies have demonstrated the importance of functional diversity in competitive

suppression of invaders (Crawley *et al.*, 1999; Hector *et al.*, 2001; Prieur-Richard *et al.*, 2002; Xu *et al.*, 2004; Fargione & Tilman, 2005). Functional groups are sets of species (not necessarily taxonomic) that show close similarities in traits related to ecosystem functioning, e.g. traits related to resource uptake and biomass production. Increasing evidence suggests that the influence of functional diversity in a community might be more important than pure species richness (Diaz & Cabido, 2001; Garnier *et al.*, 2004; Heemsbergen *et al.*, 2004; Petchey *et al.*, 2004).

It is supposed that empty niche space (Hutchinson, 1957) declines with increasing species richness in a community (MacArthur, 1970). As a consequence, species-rich communities can utilize the total resources available in a biotope more completely than do species-poor communities (e.g. Scherer-Lorenzen *et al.*, 2003; Dimitrakopoulos & Schmid, 2004), thereby pre-empting resources for potential invaders (Tilman, 1999; Hector *et al.*, 2001; Fargione *et al.*, 2003). This effect occurs because, generally, an increase in species richness should also increase functional richness, suggesting that the number of functional groups in an experimental community may be a good predictor of these diversity effects. Conversely, the effect should be minimal if species richness is increased without increasing the number of functional groups at the same time. In addition, a community should be more resistant to invaders belonging to functional groups already present among the resident species (e.g. Fargione *et al.*, 2003; Turnbull *et al.*, 2005).

In most cases, invasion studies compare a set of species used as test invaders with a separate set of species used as residents of host communities (Tilman, 1997; Knops *et al.*, 1999; Hector *et al.*, 2001; Prieur-Richard *et al.*, 2002; Fargione *et al.*, 2003; Pfisterer *et al.*, 2004, but see Turnbull et al. 2005). This approach mimics biological

invasions into communities by exotic species. For example, the average competitive ability of exotic invaders may change with diversity (e.g. Bossdorf *et al.*, 2004; Colautti *et al.*, 2004; e.g. Vila & Weiner, 2004; Hierro *et al.*, 2005). Here we do not analyze exotic species invasions but rather invasion as a process of community assembly within a pool of native species. In this case, because the host communities and invaders belong to the same species pool, it is possible to distinguish between the *invasiveness* of a particular species or functional group as an invader and its contribution to *invasion resistance* of the host community within the same experiment.

Using this approach, we selected four native species representative of four functional groups used in a biodiversity experiment as test invaders or "phytometers". Specifically, we wanted to find out: (a) if increasing species richness or number of functional groups in plant communities suppresses the performance of invaders; (b) whether the presence of a particular functional group in a host community enhances suppression of the test invaders; and (c) whether the test invaders are most suppressed by host communities containing species belonging to the same functional group.

Material and methods

Our study was part of a large biodiversity experiment, The Jena Experiment in Germany (50°55' N, 11°35' E, 130 m altitude). This experiment was established in May 2002 on a former agricultural field in the flood plain of the Saale river (Roscher *et al.*, 2004). Plant communities were assembled by constrained random selection from a pool of 60 species typical to Central European mesophilic grasslands. The species were categorized into the four functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species), based on multivariate

analyses of their traits (Roscher *et al.*, 2004). Analyzed traits included growth form (6 binary traits), lateral clonal spread, height of vegetative and flowering plant, leaf size, depth and type of root system, life cycle, seasonality of foliage, onset and duration of flowering and nitrogen fixation. Seventy-eight plots, each measuring 20 x 20 m, were sown with 1, 2, 4, 8, or 16 species. A factorial design was formed with all possible species richness x functional group richness mixtures. At each level of species richness, 16 replicate mixtures with different species composition were established, except at the highest level with 14 replicates only. Four additional large plots contained mixtures of all 60 species in the pool. The field was partitioned into four blocks following a gradient in soil characteristics perpendicular to the river (Roscher *et al.*, 2004). The plots were mowed twice a year (June, September), as is typical for this type of grassland ecosystem, and weeded twice a year to maintain the original species composition. Mowing and weeding were done block-wise such that these management effects could be accounted for with the block term in statistical analysis.

Our test invaders were pre-grown phytometer individuals of four species that also occurred in a large number of experimental communities as resident species. Clements and Goldsmith (1924) introduced the term “phytometer” for test plants that were used to measure environmental factors. Each of the four species belonged to a functional group used in the experiment: *Festuca pratensis* Huds. (grass), *Plantago lanceolata* L. (small herb), *Knautia arvensis* L. (tall herb) and *Trifolium pratense* L. (legume). They are all perennial plant species, form clearly defined compact individuals and are relatively easy to transplant.

In mid-March 2003, we germinated the phytometers on moist filter paper in a greenhouse. Individual seedlings were planted in 132-cm^3 cells of potting trays filled

with a soil-compost-perlite mixture (3:2:1 in terms of volumes), and were exposed to a 14-h light regime with 22°C day temperature and 15°C night temperatures. In mid-April 2003, most of the plants had 4–7 leaves. We placed them outside the greenhouse for hardening and one week later transplanted them into the experimental communities. Five phytometer individuals of each test species were randomly allocated to positions at 28-cm intervals in a 2 x 2-m subplot within each large plot and the initial size determined by counting their number of leaves and number of ramets (the latter only for *F. pratense* and *T. pratense*). Transplanted phytometers were marked by fixing numbered plastic labels next to the plants to ease identification during data collection.

In mid-August, in addition to counting the number of leaves, we measured the maximum height of the phytometers. For *T. pratense* and *F. pratensis,* we also counted the number of ramets as before. We calculated the relative growth rate of the transplants using the formula

$$RGR = (\ln l_{t2} - \ln l_{t1})/d,$$

where l_{t2} is the mean number of leaves in August, l_{t1} is the mean number of leaves in April and d is the length of time interval in days (Harper, 1977). As a measure of plant fitness, in August, we also counted the number of inflorescences of *P. lanceolata* and *T. pratense* transplants. No individuals of *F. pratensis* or *K. arvensis* were flowering at this time. In the last week of August 2003, shortly before mowing, transplants were cut at 3 cm above the ground and dried at 70°C for at least 48 h to determine the average biomass of each transplant species per plot. In early June 2004, we once again measured the height of the transplants, counted the number of inflorescences in *P. lanceolata* and *T. pratense,* which were flowering at this time, and harvested the transplants per species per plot to determine the average aboveground biomass as described above.

Before each harvest, we determined the leaf area index (LAI) of the resident community in an undisturbed area next to the phytometers using an LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, Nebraska, USA).

Statistical analysis

We used general linear models with sequential sum of squares (Type I) for data analysis using Genstat 6th Edition, Release 6.2. (Payne *et al.*, 2002). Since individual plants were pseudo-replicates within plots, we analysed means of the response variables plant biomass, plant height, number of leaves and relative growth rate. The data were transformed if residuals showed deviation from the normal distribution. According to the experimental design, the analysis of variance (ANOVA) model consisted of the terms block, sown species richness (partitioned into log-linear contrast and deviation from log-linear contrast), functional group richness, species composition, phytometer species and phytometer species x diversity interactions. Separate contrasts for the presence/absence of each functional group and their interactions with species richness were tested in alternative models. Similarly, separate contrasts were made to compare each phytometer species and its interactions with diversity terms against the other three phytometer species. The diversity terms (species richness, functional group richness, presence of particular functional groups) had to be tested at the between-plot level (Error = composition) whereas phytometer terms and their interactions with diversity terms could be tested at the within-plot level (Schmid *et al.*, 2002). We also analysed the data of each phytometer species separately. To determine if the effect of diversity terms was related to a change in the leaf-area index (LAI) of the community we did

post-hoc analyses with LAI as a covariate. In addition, LAI was tested as a dependent variable itself, using the between-plot ANOVA as explained above.

To test if the phytometer species were more affected by their own than by other functional groups, we used a reduced data set of communities with only one functional group (n = 34 plots). To do this, the resident x transplant functional group interaction was decomposed into a "home versus away" contrast and remainder (taking all "away" treatment combinations together; see Table 3.3). To illustrate the home versus away contrast we use an equivalent of the relative-neighbour-effect of (Markham & Chanway, 1996), using the formula $(P_h-P_a) / \max(P_h, P_a)$. Here, P_h is the performance (e.g. biomass) of phytometers in communities with their own functional group (home), P_a the performance in communities with other functional groups (away) and $\max(P_h, P_a)$ is the larger of the two.

Results

Effects of species richness and functional richness

Except for plant height in *P. lanceolata* and *K. arvensis*, the measured morphological variables of phytometers were highly correlated with their aboveground biomass (Table 3.1), indicating that the latter is a good measure of overall phytometer performance.

At the first harvest in summer 2003, i.e. 4 months after transplanting, the performance of phytometer individuals was negatively related to the logarithm of sown species richness (reduced number of leaves or number of ramets, reduced biomass and reduced growth rate, Fig. 3.1a, c, d and Table 3.2a). The height of the phytometers was, however, not affected and even increased with the logarithm of species richness in one

of the phytometer species (*F. pratensis*; $F_{1,70}$ = 9.30, p < 0.01 in separate analysis), suggesting a typical allometric response to increased competition for light (etiolation); i.e. a faster increase in height, independent of size (Fig. 3.1b, Table 3.2a; see also lower correlations of plant height than of other variables with biomass in Table 3.1). The length of the leaves in *F. pratensis* also increased with increasing species richness ($F_{1,73}$ = 15.64, p < 0.001). The influence of resident species richness on phytometer performance varied among phytometer species; the herbs (*P. lanceolata* and *K. arvensis*) were more strongly affected than the grass (*F. pratensis*) and the legume (*T. pratense*) (see species richness x phytometer species (PS) interaction in Table 3.2a). The negative effect of species richness on phytometer aboveground biomass was still significant in spring 2004 ($F_{1,73}$ = 27.80, p < 0.001, Fig. 3.2b), but again plant height was not affected by species richness ($F_{1,73}$ = 1.19, p < 0.172, Fig. 3.2a).

Table 3.1 Partial correlation of phytometer vegetative and reproductive traits measured in summer 2003 with aboveground biomass harvested at the same time. Significance levels are, * P<0.05; ** P<0.01; ** P<0.001

Plant Trait	*F. pratense* (N=71)	*P. lanceolata* (N=78)	*K. arvensis* (N=76)	*T. pratense* (N=68)
Number of leaves	0.919***	0.887***	0.855***	0.776***
Number of ramets	0.904***	-	-	0.866***
Height	0.467***	0.128^{ns}	0.228*	0.415***
Number of flowers	-	0.974***	-	0.938***
Relative growth rate	0.804***	0.610**	0.720***	0.680***

Table 3.2a Summary of analyses of variance of the performance of the four phytometer species in summer 2003. Residual d.f. are [a]=225, [b]=219, and total [c]=308 for number of leaves and plant height and [a]=240, [b]=234 and total [c]=327 for growth rate. PS (transplant functional group) is the phytometer species. Due to hierarchical design of our experiment, the terms above composition are tested at plot-level error term, i.e. composition. The terms presence of functional groups and their interaction with SR were added alternatively because they are intrinsically related, same communities contained presence/absence of different groups. PS and SR x PS was tested against within plot error (residual). Significance levels are * P < 0.05; ** P < 0.01; *** P < 0.001.

		BIOMASS			LEAVES			HEIGHT			GROWTH RATE		
Source	d.f.	MS	F		MS	F		MS	F		MS	F	
Block	3	6.23	2.63		1.06	1.23		0.79	1.61		<0.01	1.53	
Species richness (SR)	1	22.22	**9.39**	**	14.84	**17.23**	***	1.17	2.36		<0.01	**13.59**	***
Deviation	3	5.17	2.18		1.26	1.46		0.26	0.53		<0.01	0.71	
Composition	70	2.37	**3.28**	***	0.86	**4.28**	***	0.49	**4.30**	***	<0.01	**3.07**	***
Grasses (GR)	1	19.52	**9.25**	**	3.20	**3.89**	*	4.93	**11.66**	**	<0.01	**4.41**	*
SR x GR	1	2.72	1.29		1.10	1.33		0.93	2.19		<0.01	2.98	
Composition	68	2.11	**2.89**	***	0.82	**4.21**	***	0.42	**3.82**	***	<0.01	**3.11**	***
Short-herbs (SH)	1	3.33	1.41		0.15	0.17		1.06	2.19		<0.01	0.46	
SR x SH	1	1.60	0.68		0.00	<0.01		0.77	1.59		<0.01	0.99	
Composition	68	2.37	**3.39**	***	0.88	**4.36**	***	0.48	**4.13**	***	<0.01	**3.07**	***
Tall-herbs (TH)	1	4.22	1.78		1.27	1.46		0.70	1.41		<0.01	0.04	
SR x TH	1	0.06	0.02		0.08	0.09		0.31	0.62		<0.01	<0.01	
Composition	68	2.38	**3.31**	***	0.87	**4.23**	***	0.49	**4.38**	***	<0.01	**3.12**	***
Legumes (LG)	1	13.15	**5.86**	*	0.82	0.94		7.18	**17.88**	***	<0.01	<0.01	
SR x LG	1	0.03	0.01		0.35	0.41		0.08	0.20		<0.01	0.17	
Composition	68	2.24	**3.29**	***	0.87	**4.76**	***	0.40	**3.64**	***	<0.01	**3.31**	***
PS[1]	3	10.44	**14.47**	**	14.82	**73.58**	**	13.31	**115.8**	**	<0.01	**82.60**	***
SR x PS	3	2.07	**2.88**	*	0.25	1.26		0.17	1.45		<0.01	**3.08**	*
Residual	216[a]	0.72			0.20			0.11			<0.01		
Total	299[c]	1.39			0.56			0.34			<0.01		

Functional richness had no effect on the performance of the phytometers after controlling for species richness in both seasons ($p > 0.05$). By contrast, if fitted before species richness, functional richness also had significant negative effects on all phytometer variables except height (aboveground plant biomass: $F_{1,67} = 4.74$, $p = 0.03$; plant height: $F_{1,67} = 1.38$, $p = 0.24$; number of leaves: $F_{1,67} = 7.13$, $p = 0.01$; growth rate: $F_{1,67} = 6.12$, $p = 0.01$; Fig. 3.1e-h); and in addition the species richness effects remained significant ($p < 0.05$) except for plant height, as before. The pattern was the same in spring 2004 (Fig. 3.2c and d). This highlights the importance of species richness even if

functional richness in statistical terms is “held constant”, i.e. the species richness effect remains negative within a particular level of functional richness.

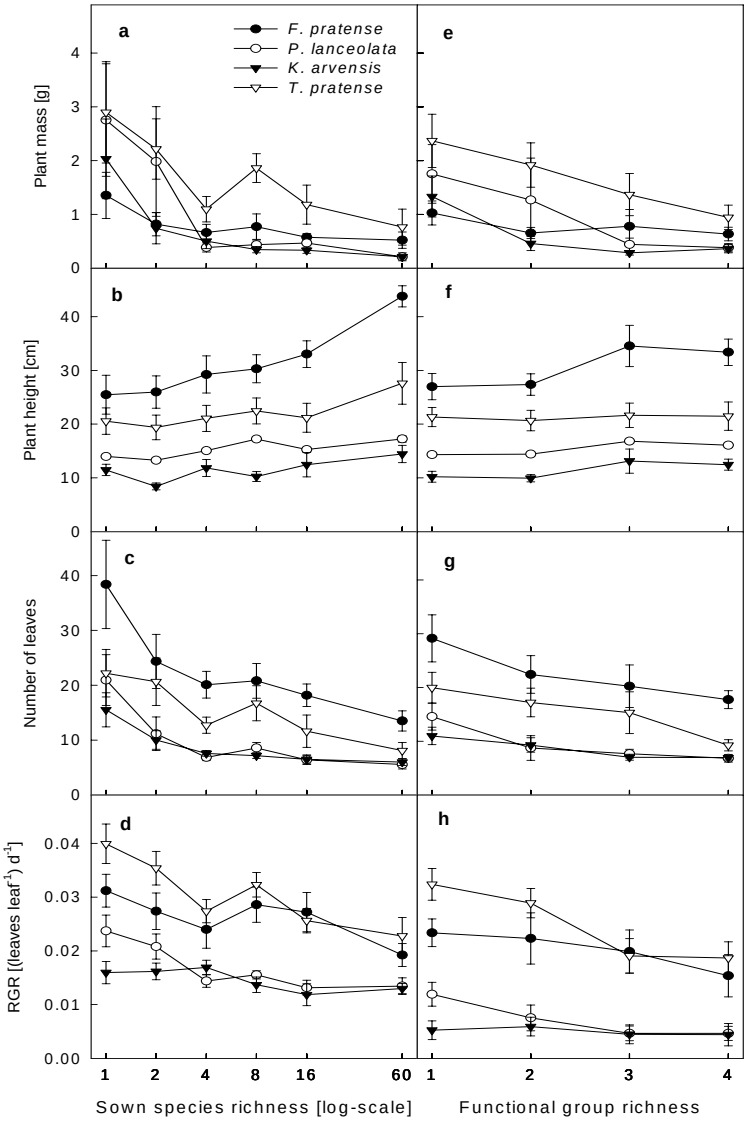

Figure 3.1 Effect of plant species richness (a–d) and functional group richness (e–h) on performance of four transplanted phytometer species in the first season (summer 2003). Points and vertical bars represent means ± 1 standard error. All panels use the legend in panel (a).

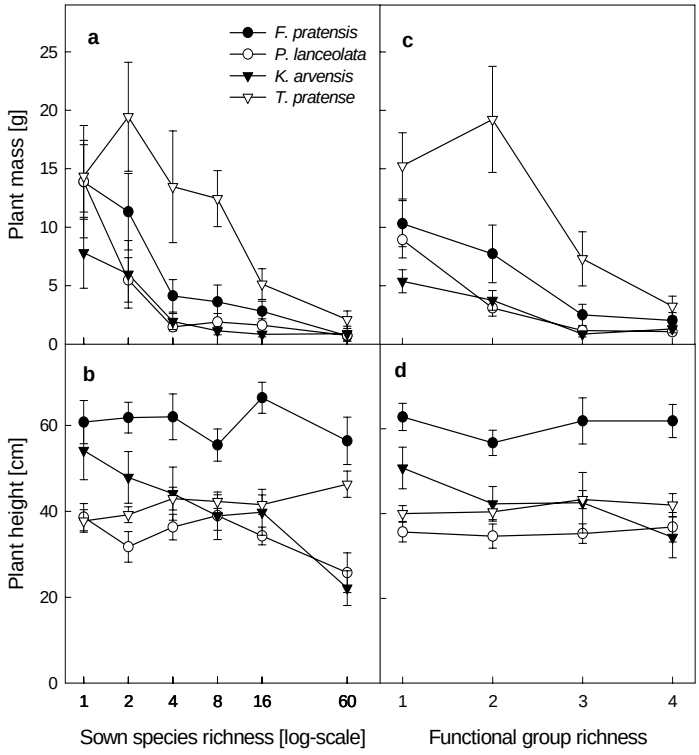

Figure 3.2 Effect of plant species richness (a–b) and functional group richness (c–d) on performance of four transplanted phytometer species in the second season (spring 2004). Points and vertical bars represent means ± 1 standard error. All panels use the legend in panel (a).

Separate analyses showed that increasing species richness (log-scale) led to a significant reduction in number of inflorescences per plant in *P. lanceolata* ($F_{1,70}$ = 25.58, $p < 0.001$) and *T. pratense* ($F_{1,69}$ = 6.07, $p = 0.01$), the two phytometer species which flowered before the first harvest in August 2003 (Fig. 3.3a). The same negative effect of species richness on number of inflorescences per plant was observed in *P. lanceolata* ($F_{1,69}$ = 18.31, $p < 0.001$) and *K. arvensis* ($F_{1,68}$ = 18.88, $p < 0.001$) in spring 2004 (Fig. 3.3a). Again, the effect of functional richness on the number of inflorescences was not significant after controlling for species richness, but it was highly significant if fitted first (summer 2003: *P. lanceolata*; $F_{1,70}$ = 11.15, $p < 0.001$, *T. pratense*; $F_{1,69}$ = 6.13, $p = 0.016$; spring 2004: *P. lanceolata*; $F_{1,69}$ = 12.04, $p < 0.001$, *K. arvensis*; $F_{1,68}$ = 14.71, $p < 0.001$; Fig. 3.3b), with the effect of species richness fitted afterwards again remaining significant ($p < 0.01$).

Table 3.2b Summary of analyses of variance of contrasts of phytometers species and their interaction with the diversity terms. Contrasts for each phytometer species were added alternatively into model in table 3.2a and tested against their respective residuals. Abbreviations not included in table 3.2a are; FP = *Festuca pratensis*, PL = *Plantago lanceolata*, KA = *Knautia arvensis*, TP = *Trifolium pratense*, for the phytometer species.

		BIOMASS			LEAVES			HEIGHT			GROWTH RATE		
Source of variation	d.f.	MS	F		MS	F		MS	F		MS	F	
FP	1	<0.01	<0.01		33.52	**171.4**	**	25.57	**231.06**	**	<0.01	**36.33**	***
SR x FP	1	3.55	**4.86**	*	0.42	2.14		0.50	**4.49**	*	<0.01	2.26	
GR x FP	1	0.19	0.26		1.46	**7.48**	**	0.01	0.08		<0.01	**18.02**	***
GR x PS	2	0.23	0.32		0.52	2.68		0.34	**3.04**	*	<0.01	**3.89**	*
SR x GR x FP	1	0.38	0.52		0.00	0.02		0.15	1.31		<0.01	2.26	
SR x GR x PS	2	0.64	0.88		0.00	0.00		0.40	**3.57**	*	<0.01	0.13	
Residual	210[b]	0.73			0.20			0.11			<0.01	0.61	
PL	1	1.49	2.13		12.46	**61.43**	**	2.15	**18.44**	**	<0.01	**50.16**	***
SR x PL	1	3.68	**5.27**	*	0.53	2.60		0.04	0.34		<0.01	0.77	
SH x PL	1	1.67	2.39		0.29	1.43		0.05	0.46		<0.01	0.15	
SH x PS	2	2.06	2.96		0.06	0.28		0.10	0.84		<0.01	0.66	
SR x SH x PL	1	2.01	2.87		0.00	0.01		0.00	0.01		<0.01	0.86	
SR x SH x PS	2	0.66	0.94		0.24	1.20		0.03	0.28		<0.01	0.93	
Residual	210[b]	0.70			0.20			0.12					
KA	1	14.31	**19.96**	**	12.03	**58.72**	**	23.91	**212.05**	**	<0.01	**107.17**	***
SR x KA	1	0.56	0.78		0.02	0.11		0.08	0.71		<0.01	3.49	
TH x KA	1	0.00	0.00		0.01	0.04		0.18	1.56		<0.01	0.17	
TH x PS	2	1.69	2.35		0.15	0.74		0.10	0.89		<0.01	0.75	
SR x TH x KA	1	0.02	0.02		0.01	0.03		0.37	3.31		<0.01	0.56	
SR x TH x PS	2	0.87	1.22		0.07	0.33		0.21	1.84		<0.01	0.45	
Residual	210[b]	0.72			0.20			0.11					
TP		25.83	**37.88**	**	1.38	**7.56**	**	1.56	**14.17**	**	<0.01	**144.96**	***
SR x TP	1	0.56	0.82		0.05	0.28		0.05	0.49		<0.01	**6.25**	*
LG x TP	1	8.17	**11.97**	**	2.56	**14.02**	**	0.44	**3.97**	*	<0.01	**7.61**	**
LG x PS	2	0.76	1.11		0.82	**4.50**	*	0.06	0.51		<0.01	2.35	
SR x LG x TP	1	0.55	0.81		0.16	0.89		0.54	**4.89**	*	<0.01	0.77	
SR x LG x PS	2	1.16	1.71		0.45	2.45		0.31	2.78		<0.01	2.53	
Residual	210[b]	0.68			0.18			0.11			<0.01		

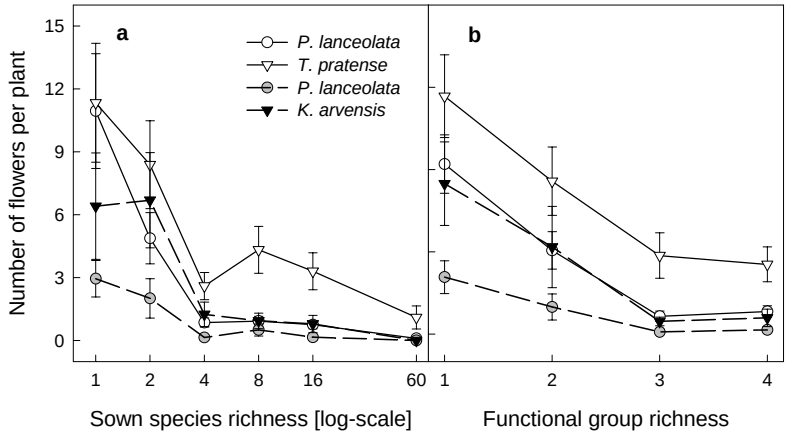

Figure 3.3 Effect of plant species richness (a) and functional groups richness (b) on number of inflorescences of four transplanted phytometer species in summer 2003 (solid lines) and spring 2004 (broken lines). Points and vertical bars represent means ± 1 standard error.

Effects of the presence of particular functional groups

The presence of grasses or legumes in the host communities had significant overall effects on phytometers, but this was not the case for the other two functional groups (Table 3.2a). Grasses significantly reduced number of modules (number of leaves or number of ramets), aboveground biomass, and growth rate of all the phytometer species (Table 3.2a) as well as number of inflorescences in *P. lanceolata* in summer 2003 ($F_{1,68}$ = 5.66, p = 0.02) and spring 2004 ($F_{1,67}$ = 7.56, p = 0.008). For example, in summer 2003, the average biomass of an individual phytometer (all species together) was 0.8 g in plots with grasses compared to 1.9 g in plots without grasses (Fig. 3.4). In spring 2004, the figures were 3.5 g and 10.8 g for plots with and without grasses respectively. The presence of legumes had an overall significantly positive effect on the performance of the phytometer species (Table 3.2a, Fig. 3.4 and 3.5). Separate analysis for each phytometer, however, revealed that the presence of legumes actually reduced aboveground biomass of the legume phytometer, *T. pratense*, at least in the spring 2004 ($F_{1,61}$ = 8.97, p = 0.004). The negative effect of legume presence on the legume phytometer as opposed to a positive effect on the other phytometers is also evident in the significant contrast interaction legume presence x *T. pratense* (LG x TP in Table 3.2b) and when inspecting the last rows in figure 3.4 and figure 3.5.

There were no significant interactions between species richness and the presence of particular functional groups in the communities on phytometer performance. We mention this explicitly because such interactions might be expected if the sown proportion of a functional group would influence invasion resistance; where present, the proportion of a functional group decreases with increasing species richness.

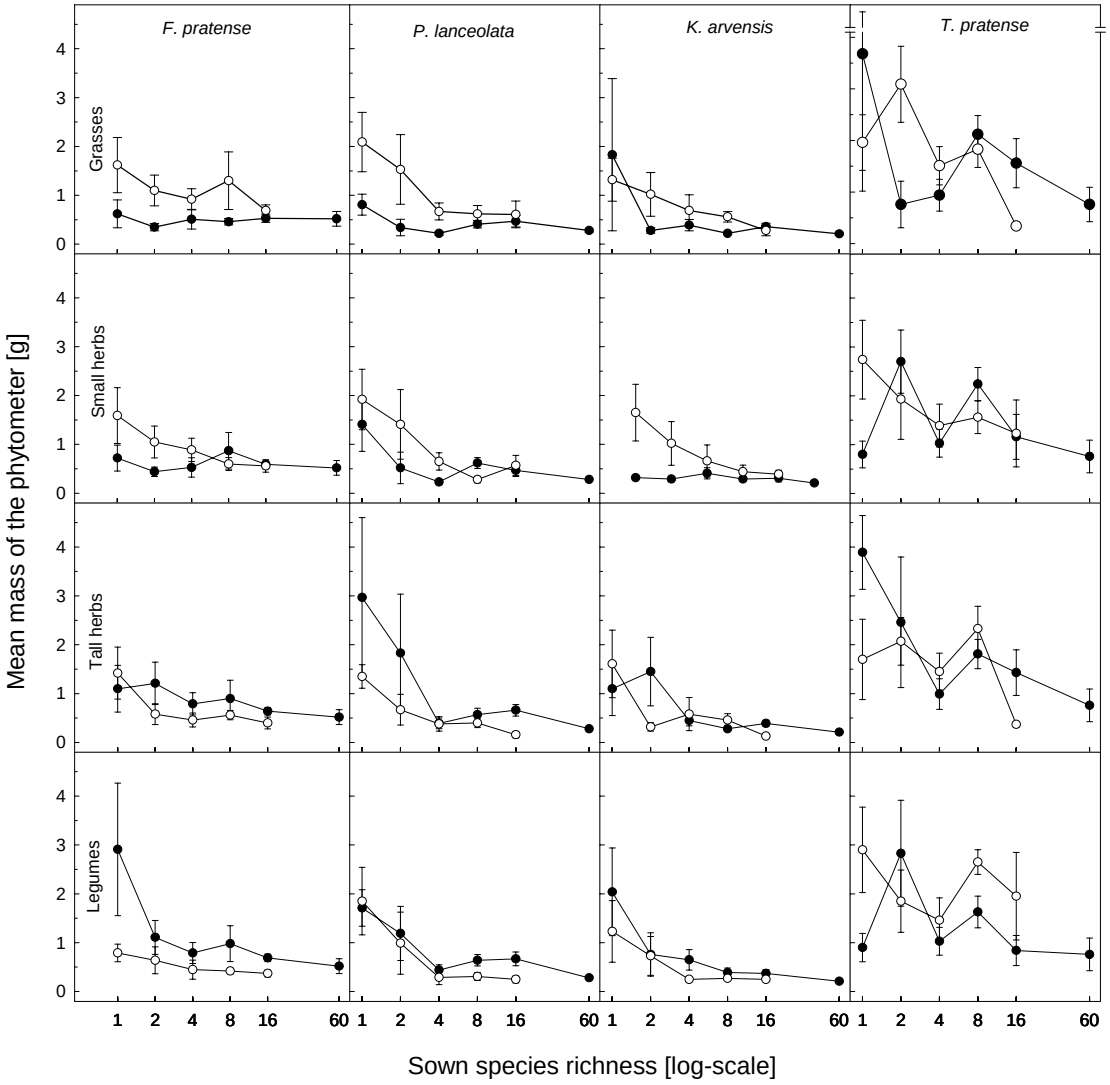

Figure 3.4 Effect of plant species richness and presence/absence of different functional groups on biomass of four transplanted phytometer species in the first season (summer 2003). Columns represent phytometers species *F. pratensis*, *P. lanceolata*, *K. arvensis* and *T. pratense* from left to right and rows represent presence/absence of grasses, small-herbs, tall-herbs and legumes from top to bottom. Closed and open symbols indicate, respective-ly, presence and absence of the corresponding functional groups in the resident communi-ties. For example, the second panel in the first row shows the response of *P. lanceolata* to species richness in the plots containing grasses (closed symbols) and in plots without grasses (open symbols). Points and vertical bars represent means ± 1 standard error.

In both seasons, the leaf area index (LAI) of the resident community increased with the logarithm of species richness (August 2003: $F_{1,68} = 6.42$, $p = 0.014$, May 2004: $F_{1,70} = 6.70$, $p = 0.012$) but was not affected by functional richness. Although there was

no effect of the presence of any functional group on LAI in August 2003, in May 2004, LAI was high in mixtures containing legumes ($F_{1,68}$ = 28.01, p < 0.001) and low in mixtures containing small herbs ($F_{1,68}$ = 6.52, p < 0.013). This suggests that belowground competition may be responsible for the observed high suppression of phytometers in communities containing grasses. As a covariate, in August 2003, LAI had significant negative effects on number of leaves and growth rate, positive effects on plant height (P < 0.05) but neutral effects on biomass of the phytometers. In May 2004 however, LAI had negative effects on aboveground biomass, plant height, and number of ramets of the phytometers (P < 0.05). However, where present, the effects of LAI did not explain the significant effects of species richness; that is, species richness effects remained significant after controlling for the effect of LAI.

Effects of the functional group of the phytometer species

As suggested by hypothesis (c) in the Introduction, comparing the suppression of invaders by communities containing different functional groups is not the same as looking at the performance of invaders belonging to different functional groups. In the first case (hypotheses (a) and (b) in the Introduction), the panels in Fig. 3.4 and Fig. 3.5 are compared row-wise, in the second case they are compared column-wise. If the two approaches are combined, the performance of particular phytometer species in assemblages containing only species of its functional group can be compared with its performance in assemblages containing only the other functional groups (–1 diagonal in Fig. 3.4 and Fig. 3.5). We refer to this as a "home-vs.-away" contrast (see e.g. Joshi *et al.*, 2001; Turnbull *et al.*, 2005), for which hypothesis (c) predicts a particularly strong negative effect.

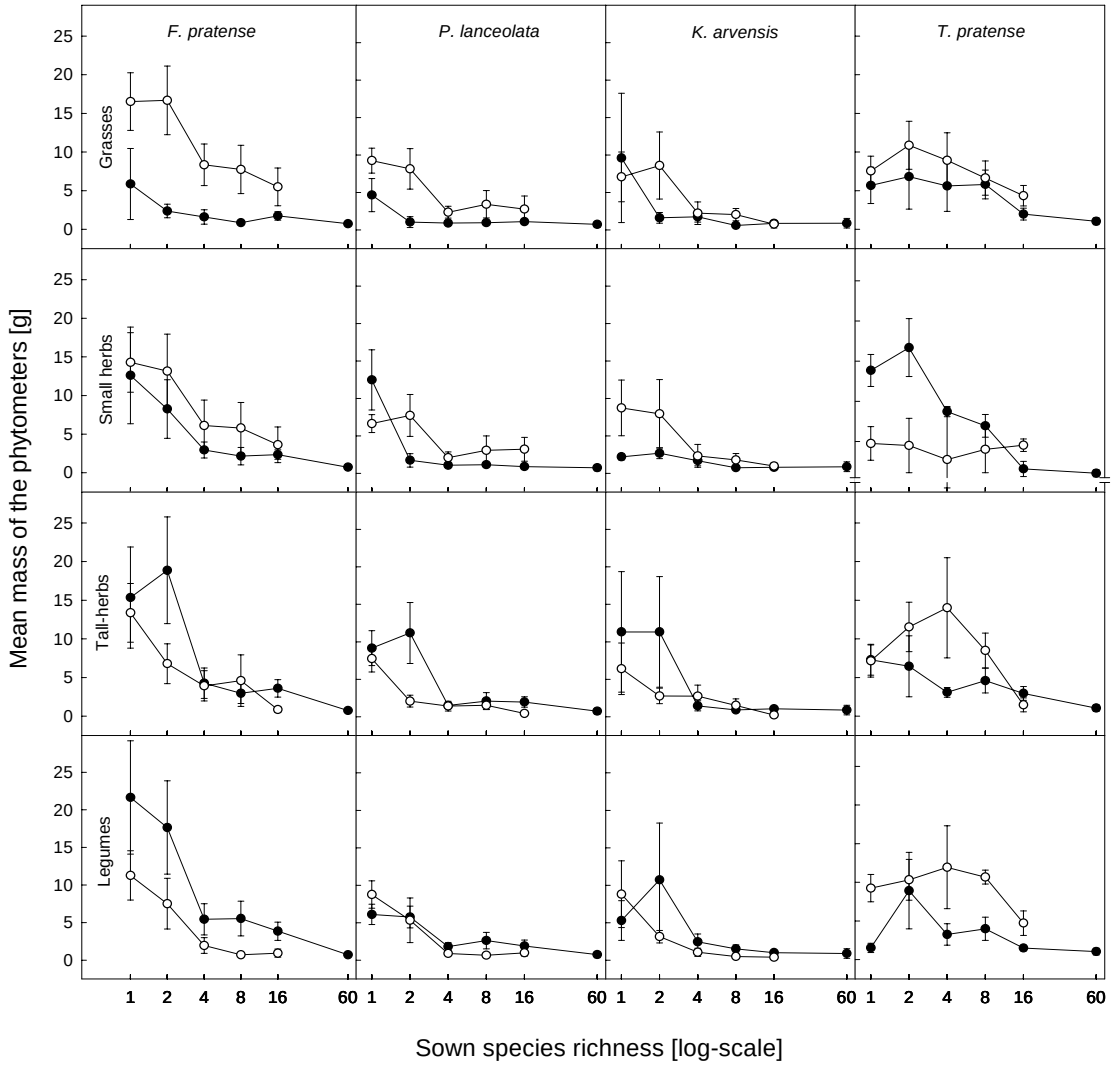

Figure 3.5 Effects of plant species richness and presence/absence of different functional groups on biomass of four transplanted phytometer species in the second season (spring 2004). Columns represent phytometers species *F. pratensis*, *P. lanceolata*, *K. arvensis* and *T. pratense* from left to right and rows represent presence/absence of grasses, small herbs, tall herbs and legumes from top to bottom. Closed and open symbols indicate, respectively, presence and absence of functional groups in the resident communities. Points and vertical bars represent means ± 1 standard error.

The effect of different single-functional-group assemblages on number of modules (leaves or ramets), aboveground biomass and growth rate of the phytometers was similar (Table 3.3). However, the height of the phytometers significantly differed among these assemblages: it increased from grass < small-herb < tall-herb < legume communities, suggesting that competition for light increased in this order. Overall, the

two herbaceous phytometer species were least affected by differences between these one functional group assemblages, whereas the grass (*F. pratensis*) and the legume (*T. pratense*) phytometer were more affected by these differences. This is evident in Fig. 3.5 by comparing differences between open and filled symbols in monocultures. A contrast between monocultures versus multi-species assemblages containing one functional group showed that the number of modules (leaves or ramets), aboveground biomass and growth rate of the phytometers was significantly lower in the latter (Table 3.3). This reinforces the statistical observation made above, that competitive suppression increases with species richness of a community even if functional richness is held constant, in this case at the lowest level. The home-disadvantage was similar in mono-specific and multi-species single-functional-group assemblages (interaction home x mono not significant in Table 3.3).

Table 3.3 Summary of analyses of variance of summer 2003 data for home-vs.-away effect on the four phytometer species using plots with resident communities consisting of species from only one functional group. Significance levels are ***** $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

		Biomass		Leaves		Height	
Source	d.f.	MS	F	MS	F	MS	F
Block	3	11.63	**4.09***	2.33	2.08	553.61	8.43
Resident Functional Group (RFG)	3	3.11	1.09	0.26	0.23	962.34	**14.66*****
Monoculture vs. Mixture (Mono)	1	22.02	**7.75***	9.16	**8.19****	49.64	0.76
RFG x Mono	3	0.53	0.19	0.06	0.06	6.44	0.10
Composition	23	2.84	**4.19*****	1.12	**5.61*****	65.66	**1.68***
Phytometer species (PS)	3	3.36	**4.95****	5.99	**30.08*****	1818.79	**46.65*****
Home vs. Away (RFG x PS main diag.)	1	4.25	**6.26***	1.59	**7.98****	169.93	**4.36***
RFG x PS (residual interact.)	8	0.84	1.24	0.22	1.13	191.49	**4.91*****
Phytometer species x Mono	3	2.47	**3.64***	0.28	1.40	42.37	1.09
Home vs. Away x Mono	1	0.19	0.28	0.38	1.89	8.51	0.22
Residual	79	0.68		0.20		38.99	
Total	128	1.68		0.63		128.91	

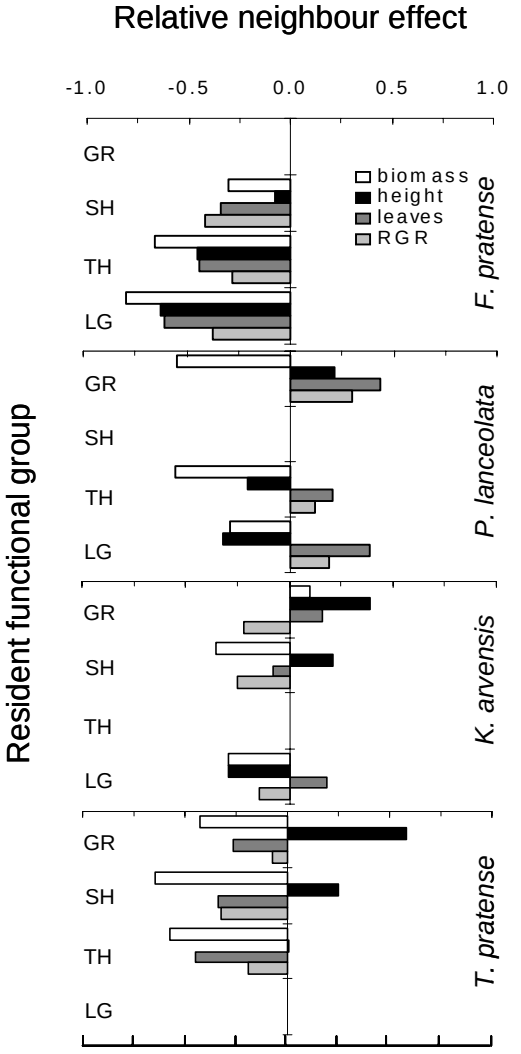

Figure 3.6 Performance of phytometers transplanted into single-functional-group assemblages of grasses (GR), small herbs (SH), tall herbs (TH) and legumes (LG) relative to performance in mono-functional group assemblages of their own functional group. Negative values indicate negative "home" effects, i.e. that resident species belonging to a functional group different from that of the phytometer were less inhibitive, while positive values indicate positive home effects.

Except for plant height, the home vs. away contrast almost fully explained the resident functional group x phytometer species interactions (Table 3.3). That is, as predicted, the phytometers had significantly lower performance when transplanted into assemblages consisting of the same rather than a different functional group (the effects of home-functional groups were stronger than of away-functional group, i.e. negative bars in Fig. 3.6). The significant residual RFG x PS interaction for plant height indicates that the home effect on plant height is not as clear-cut; for example, *F. pratense* phytometers were taller in non-grass single function group assemblage whereas *T.*

pratense phytometers were shorter in non-legume single functional group assemblages (Fig. 3.6). The four-phytometer species responded differently to mono-specific versus multi-species single-functional-group assemblages (Table 3.3): the biomass of *P. lanceolata* declined from 5.3 g in mono-specific to 0.9 g in multi-species single-functional-group assemblages whereas the other three-phytometer species showed little reduction in aboveground biomass. For two phytometer species that also occurred as monocultures, *P. lanceolata* was greatly suppressed by its own monoculture in both seasons. It attained less than 1.5 g in its own monoculture in both seasons compared to an average of 5.6 g and 14.7 g in other monocultures in summer 2003 and spring 2004 respectively. By contrast, *F. pratense* performed well in its own monocultures especially in 2004 (i.e. 19.6 g in its own and 13.5 g in others).

Discussion

Effects of species richness and functional richness

By introducing young plants of native species as test invaders into host communities we have shown that plant diversity enhances competitive suppression of newly arriving individuals during the invasion process. This supports the proposition that species-rich communities contain less empty niches that can be occupied by extra individuals. The performance of the test invaders in our experiment was linearly related to the logarithm of species richness. This indicates an attenuation of invasion resistance in more diverse host communities, probably due to increased niche overlap among resident species. It is conceivable that stronger invasion resistance could have been observed if we had introduced seeds (see e.g. Symstad, 2000; Dukes, 2001) instead of young plants, but then we would have confounded requirements for empty germination niches with those

for growing plants. Our results agree with several previous findings referring to invasions by non-native species (e.g. Levine, 2000; Prieur-Richard *et al.*, 2002). With one of the most balanced designs in terms of species and functional diversity achieved so far in biodiversity experiments (Roscher *et al.*, 2004), our results show that in contrast to previous suggestions (Diaz & Cabido, 2001) species richness was a better predictor of invasion resistance than was functional group richness.

Since functional groups are aggregations of species, three observations (that can apply in natural communities) may explain why the effect of species richness in this study was stronger than that of functional group richness. First, aggregating several species into few functional groups makes species richness have a wider range (1–60 species) than functional richness (1–4 functional groups). Second, differential effects of functional richness on different phytometer species leads to an averaging of the overall effect of functional richness in a balanced design, where each functional group is represented equally among the four phytometer species and among the resident plant communities. A case in point is the positive effect of resident legumes on non-legume test-invaders and the negative effect on the legume test-invader. Third, contrasting effects of different functional groups on resources may weaken the overall effect of functional group richness. For example, while legumes enrich soil with nitrogen, grasses deplete this resource (Tilman *et al.*, 1997; Scherer-Lorenzen *et al.*, 2003). These counteracting effects of functional groups on resource pre-emption weaken the overall effect of functional group richness on suppression of test-invaders, supporting resource pre-emption as one mechanism of invasion resistance in plant communities.

Nonetheless, as in the study by Symstad (2000), using seeds instead of transplants as invaders, functional group richness can enhance invasion resistance, if

considered alone. Interestingly, Symstad (2000) could not attribute increased invasion resistance by functionally rich communities to resource pre-emption, which indicates that in her case perhaps specific requirements for germination played an important role. Our study showed that pure grass mixtures can be most resistant to invasion after germination, underlying the importance of traits of specific groups (see next section). It remains debatable, of course, if the *a priori* definitions of functional groups that we adopted in The Jena Experiment are adequate to understand the relationship between functional diversity and invasion resistance. Nevertheless, the fact that species richness log-linearly increased invasion resistance even within plant assemblages consisting of a single functional group further exemplifies the importance of species richness as a driver in this particular case of an ecosystem function.

Effects of the presence of particular functional groups

The negative, positive and neutral effect of legumes, grasses and herbs, respectively, on invasion resistance in our study reflects their known patterns of resource use (Fargione *et al.*, 2003; Fargione & Tilman, 2005) and fits with a general theory of invasibility of Davis *et al.* (2000). In our experiment, better performance of the phytometers in communities with legumes corresponded with findings that they actually benefited from nitrogen fixed by legumes (Temperton *et al.*, submitted). It is well documented that legumes, by adding nitrogen to the soil, can promote invasion in nitrogen-limited environments (Yelenik *et al.*, 2004 and reference therein). By actively fixing atmospheric nitrogen, legumes do not rely on soil-nitrogen pools. Thus, a related effect of legumes is the reduction of competition for soil nitrogen. Some previous studies, however, have reported increased invasion resistance due to presence of legumes

(Hector *et al.*, 2001; Fargione *et al.*, 2003). It is notable that positive effects of legumes usually correlate with their effect on belowground resources, mainly soil nitrogen (Maron & Connors, 1996; Prieur-Richard *et al.*, 2002) while their negative effects usually correlate with their effect on aboveground resources (Hector *et al.*, 2001; Fargione *et al.*, 2003). Thus, in general legumes may enhance invasion resistance in fertile soils but promote invasion in poor soils. In addition, legumes may have a stronger potential than grasses and herbs to differentially affect different invaders. Notably, as mentioned above, they can have negative effects on other legumes even when they have positive effects on other species.

Suppression of all phytometers was particularly strong in resident communities containing grasses. Due to their extensive root systems, grasses are efficient in taking up resources from the upper soil layers (Fargione *et al.*, 2003), thereby diminishing resources for potential invaders. Other studies have also reported grasses as a keystone functional group reducing the success of invaders (Crawley *et al.*, 1999; Dukes, 2002; Prieur-Richard *et al.*, 2002). Crawley *et al.* (1999) found that an assembly of 80 herbaceous species was more vulnerable to invasion than were assemblies composed of 1–4 grass species. A weak effect of grasses on LAI did not explain the strong negative effect of their presence on invasion resistance, suggesting that their contribution to invasion resistance is mainly through their effect on belowground resources. From these results we can conclude that with regard to functional diversity, functional group identity may be more important than pure number of functional groups (Schmid *et al.*, 2002). This was also observed in the same experiment by Scherber *et al.* (2006), investigating herbivory on a different phytometer species, *Rumex acetosa*.

Effects of the functional group of the phytometer species and niche pre-emption within functional groups as major mechanism of invasion resistance

With regard to the identity of the invader, experimental communities were particularly resistant to a phytometer species if they contained species belonging to the same functional group. For example, although non-legume herbs had no effect on invasion resistance in general, their presence in the resident communities enhanced suppression of their respective phytometers. Likewise, despite notable facilitation by legumes, communities containing only this functional group strongly inhibited the legume test invader, *T. pratense*. Our results and a previous observation that legume monocultures were most resistant to invasion by legumes (Turnbull *et al.*, 2005) indicate that resident legumes also pre-empt other resources that limit legumes, most likely phosphorus, water and light (Vitousek & Howarth, 1991). This is consistent with high niche overlap along several resource-use axes between resident and invading legumes, and further supports niche pre-emption as a mechanism of invasion resistance. Thus, high niche overlap between newly arriving individuals and resident species can reduce chances of an invasion, rate of colonisation or even success of restoration. This corresponds to findings of Fargione *et al.* (2003) in a seed addition experiment, where they concluded that high invasion resistance was due to similar patterns of resource use between the resident species and the invaders. Xu *et al.* (2004) also found that the presence of a functionally similar herb in a resident community increased resistance to invasion by Alligator weed (*Alternanthera philoxeroides*), which was also attributed to niche overlap.

Finally, it should be mentioned that alternative hypotheses may also be consistent with the result of stronger within- than between-functional-group invasion

resistance (Fukami *et al.*, 2005; Britton-Simmons, 2006). Namely, the presence of a species in a community might build up pathogens, parasites, or herbivores that have negative impacts on invaders from the same functional group (Wardle *et al.*, 2004; Bartelt-Ryser *et al.*, 2005). It is conceivable that such effects are particularly important during the early phases of the life cycle of invaders, e.g. during germination. However, in this study we could not test this because we introduced our test invaders as young plants rather than seeds into the communities.

This study confirms that, first, communities that are more diverse confer high resistance to invasion independent of invasiveness of the introduced species. Secondly, presence of grasses enhances invasion resistance while legumes may promote invasion due to their influence on nitrogen dynamics. Thirdly, communities are more resistant to invaders belonging to functional groups already present among the resident species. Although these results pertain to grassland ecosystems which are regularly "disturbed" by mowing, similar mechanisms may play a role in other terrestrial and in aquatic ecosystems where species richness has been shown to increase invasion resistance (see review of Balvanera *et al.*, in press). However, to our knowledge this has not been investigated in such a systematic way as we did here in grassland systems.

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Chapter 4

Removing Aboveground Competition does not Reduce Negative Biodiversity Effects on *Knautia arvensis* Transplants

Manuscript

Together with M. Schmitz, M. Scherer-Lorenzen, W.W. Weisser, B. Schmid

Abstract

Newly arriving plants are more competitively suppressed in plant communities that are more diverse because they use resources more exhaustively than species-poor communities do. However, the relative contributions of root and shoot competition in this process are still unknown. We compared the performance of *Knautia arvensis* transplants growing in full competition and with shoots of neighbours tied back to determine the relative strength of aboveground competition in suppressing this test invader without the confounding effect of shading. Knautia could grow in all the studied communities if seeded at the same time as all other species. Aboveground biomass, height, leaf mass per area (LMA) and flowering of transplanted individuals of *K. arvensis* decreased with increasing species richness of the host community. Removing the aboveground competition did not reduce the suppressive effects of species richness on the transplants biomass, suggesting that the species richness effect was mainly through belowground competition. The transplants with aboveground competition were taller and had lower LMA than those without aboveground competition and this shade-avoidance strategy allowed them to maintain the same biomass. The transplants with removed shading had a lower (54.2) LMA than plants grown in monocultures (63.6). In addition to species richness, the presence of grasses in a plant community further reduced the biomass of transplants of *K. arvensis*, whereas the presence of legumes increased it and the presence of non-legume forbs had a neutral effect. Our results suggest that root competition, in particular by grasses, can render species-rich host communities more competitive suppressive to newly arriving species, thus enhancing community invasion resistance.

Keywords:

Competitive suppression, grasses, herbs, invasion resistance, legumes, shoots-exclusion, species richness, transplant performance

Introduction

Increasing evidence shows that the chances of establishment of newly arriving plants in grassland communities decreases with increasing plant diversity (e.g. Burke & Grime 1996; Tilman 1997; Crawley *et al.* 1999; Hector *et al.* 2001; van Ruijven *et al.* 2003; Fargione & Tilman 2005). Several authors attribute this phenomenon to more efficient exploitation of resources by diverse than by species-poor communities (Tilman *et al.* 1996; Hooper 1998; Tilman 1999; Naeem *et al.* 2000; Spehn *et al.* 2000; Loreau & Hector 2001; Symstad & Tilman 2001; Fridley 2003; Scherer-Lorenzen *et al.* 2003). Newly arriving plants therefore find progressively fewer "empty" niches as plant diversity increases (Elton 1958; Levine & D'Antonio 1999) and at local scale, communities become saturated with species (Cornell & Lawton 1992). For similar reasons, the presence of particular plant functional groups can affect the success of species additions to plant communities (Fargione *et al.* 2003, Mwangi *et al.* submitted). However, there are also species-poor communities, which are not invaded, and this requires additional mechanisms of invasion-avoidance, besides species richness.

Diversity-invasion resistance relationship is often explained by the relationship between the remaining below- or aboveground resources and biomass or number of invading species or individuals. In most cases, however, belowground and aboveground plant interactions are not experimentally separated in diversity-invasion resistance studies. Increasing species richness may differentially affect above- and belowground biomass production (see e.g. Spehn *et al.* 2005), which mediates the effects of plant diversity, consequently, the corresponding competition effects due to diversity or growing capacity of specific species. The magnitude of aboveground and belowground competition can

also vary differentially along biotic environmental gradients (see reviews by Wilson 1988; McPhee & Aarssen 2001).

Aboveground disturbance can reduce the suppression of invaders by diverse communities especially in the recruitment stage (Hobbs & Huenneke 1992; Burke & Grime 1996; Levine 2000; Diemer & Schmid 2001; Gross *et al.* 2005), which is particularly susceptible to aboveground space and light limitation. In contrast, after initial establishment the growth of a newly added individual in a plant community may no longer be suppressed by aboveground competition. We tested this in experimental plant communities ranging from 1–16 (60) species to which we added transplants of a phytometer species, *Knautia arvensis.* Once the phytometers had established, we reduced aboveground competition around half of them by tying back any shading tiller of the neighbours and compared their growth and aboveground biomass with the controls still experiencing full above- and belowground competition. We tested the following specific hypotheses:

a) suppression of *K. arvensis* transplants increases with increasing diversity of the plant community,
b) in addition to diversity, the presence of particular functional groups can negatively (grasses) or positively (legumes) affect the growth of transplants,
c) the suppression of *K. arvensis* transplants can be reduced by reducing aboveground competition,
d) some monocultures are particularly suppressive to the test invader,
e) morphological plasticity in response to aboveground competition may explain the response observed in (c).

Like in most previous similar studies, we could only assess the aboveground performance of the test invader. In many cases, shoot biomass is a good predictor of overall plant

performance (Howard & Goldberg 2001) although there are pros and cons when used across an environmental gradient (Cahill 2002b). In plants, morphological plasticity in response to aboveground competition is a well-documented phenomenon (e.g. Huber 1996, Huber *et al.* 1998). To assess such morphological responses, we measured plant height, leaf mass per area, leaf nitrogen concentration and the probability to bolt (produce stems) or flower on our transplants.

Materials and methods

This study was carried out in a grassland biodiversity experiment that was established in spring 2002 in Jena, Germany (50° 55' N, 11°35' E, 130m altitude, "The Jena Experiment", http://www.the-jena-experiment.de). The field site lies in the flood plain of the river Saale and it was formerly used for agricultural production (Roscher *et al.* 2004). Due to a gradient in soil characteristics, the field site was divided into four blocks, perpendicular to the river. Plant assemblies with 1, 2, 4, 8 and 16 species were formed by independent random draws with replacement from a pool of 60 naturally co-occurring central European grassland species. In addition, four plots were sown with all the 60 species. The assemblies were established on 20 x 20-m plots at a sowing density of 1000 viable seeds per square metre. There were 16 replicates with different species composition at each richness level, except at the 16-species richness level with only 14 replicates. The plant species were classified into four functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species) and legumes (12 species) based on multivariate analysis of species traits related to ecosystem functioning (Roscher *et al.* 2004). In addition, to the gradient in species richness, the number of plant functional groups was also manipulated, with 1, 2, 3, and 4 groups. Species that were not sown on a given plot

and other weeds were removed by manual cutting at ground level to maintain the designed species richness and composition treatments. At the end of May and August each year, the plots were mown and plant materials removed to mimic usual pasture management. Plots did not receive any fertilization.

In April 2003, we transplanted pre-grown *Knautia arvensis* L. as test invaders into the experimental plots. *Knautia arvensis* is a perennial herb native to Eurasia, occurring in natural and semi-natural grasslands. It is invasive in other parts of the world like North America and Canada. It has a well-developed taproot, opposite feather-shaped leaves and it can attain a height > 1 m, and therefore falls into our functional group of tall herbs in this experiment (Roscher *et al.* 2004).

In each experimental plot, we planted five randomly selected plants in a row at 28-cm intervals in a 2 x 2-m subplot of the main plot. The plants were initially grown in a glasshouse for one month and hardened at the field site for one week before transplanting. In August 2003, we counted the number of leaves of each plant and harvested the aboveground parts before routine mowing of the experimental plots. Since the plant aboveground biomass was strongly correlated with number of leaves per plant in August 2003 (r^2=0.86, n=76, p<0.001), we used the latter as a surrogate of size before application of the shoots exclusion treatment.

Exclusion treatment

Before the start of the second growing season of the transplants in April 2004, we reduced the aboveground competition around two randomly selected but adjacent *K. arvensis* transplants with a neighbouring shoot exclusion treatment. Two other transplants were used as controls (although sometimes the fifth plant was present, it was not considered for this study). Exclusion of shoots of the neighbours was achieved by installing a 30 x 60-cm

wire-mesh (mesh size 1 x 1 cm) with two holes that were 28 cm apart (i.e. the distance between two adjacent transplants) to allow unhindered growth of the target *K. arvensis* transplants. The wire-mesh was secured with two nails at about 5 cm above the ground and slightly raised on the sides to allow otherwise free growth of the neighbours that were tied back. Shoots of the neighbouring plants that would grow through the wire-mesh were pulled back under the wire mesh at weekly intervals. This exclusion treatment was nested within plots. The between-plot treatments included species richness, functional group richness and presence/absence of the four particular functional groups in the experimental assemblages (Roscher *et al.* 2004).

In the last week of May 2004, at the peak of growth, the leaf area index (LAI) between the treatment and control transplant pairs was measured by taking one measurement above the vegetation and three measurements on the ground using the LAI-2000 Plant Canopy Analyser (LI-COR Inc., Lincoln, Nebraska, USA). The LAI data were recomputed with FV200 Software (LI-COR Inc.) to restrict the measure to a 46-degree cone (two centre rings). This was considered the optimal size that did not extend beyond the exclusion area around the transplanted *K. arvensis* test individuals.

We then measured the height, noted whether the transplant had flowered and randomly harvested ten mature leaves from each pair of transplants. The leaves were transported to the lab in a cool-box where total leaf area was measured using the LI-3100 area meter (LI-COR Inc., Lincoln, Nebraska, USA). At the same time, the pairs of transplants were harvested and separated into stems and leaves (including the leaf samples) and then dried to a constant weight at 70 °C for biomass. We computed leaf mass per area (LMA) by dividing the total leaf mass by the total leaf area of each ten-leaf sample. The leaf samples were also analysed for nitrogen content.

Statistical analysis

We used analysis of variance (ANOVA) to test treatment effects on aboveground plant biomass, plant height, LMA and leaf nitrogen concentration (% of dry mass) of transplants and analysis of deviance (ANDEV) to test treatment effects on bolting and flowering probability of transplants. ANOVAs and ANDEVs were calculated with generalized linear models (Genstat 6 statistical software release 6.2, Payne *et al.* 2002). Test statistics were *F*-values (variance ratios) or quasi *F*-values (deviance ratios). Plant height and flowering probability were measured on individual transplants, all other variables were measured on pairs of transplants (and divided by two to obtain mean values for individuals).

Because of hierarchical block / plot / plant pair / plant individual design, the treatment terms in our statistical model were tested at different error strata (Schmid *et al.* 2002). Sown and realized diversity levels were very closely correlated in our experiment (Roscher *et al.* 2004) and we therefore used sown species richness and sown functional group richness to analyse diversity effects. These richness terms, each partitioned into linear contrast and deviation, were tested against plots, which represented different species compositions within richness levels. Contrasts for the presence vs. absence of each of the four functional groups and their interaction with linear species richness were fitted in turn in separate analysis runs. These contrasts were also tested at the plot stratum. The removal of aboveground competition vs. control was tested against the plant pairs as a split-plot treatment. The removal x species richness interaction, decomposed into linear contrast and deviation interaction was also tested at the plant pairs stratum. In addition, the interaction between removal and the contrast for the presence vs. absence of grasses in plant communities was fitted at this stratum. Finally, we used the community leaf area

index (LAI) and the size of transplant before the exclusion treatment as covariates to see if this could explain any of the other effects, that is, if for example increased species richness suppressed the test invader via an increased LAI.

Results

Effects of diversity

Increasing species richness had a strong negative effect on the aboveground biomass of *K. arvensis* transplants. On average, the transplants were over eight times heavier in monocultures (8.5g, s.e. 2.4) than in 16-species mixtures (1.0g, s.e. 0.32) (Fig. 4.1, Table 4.1). Similarly, the leaf mass per area (LMA) was negatively affected by increasing species richness (Fig. 4.1, Table 4.1). The transplants also showed a slight but non-significant decrease in height with increasing species richness. These responses of the transplants correlate negatively with a general increase in the leaf area index (LAI) of their neighbours with increasing species richness (Fig. 4.2, see details below), indicating that diverse host communities had a more complete aboveground space filling than did species-poor host communities. Nevertheless, transplants in *Festuca pratensis*, *Festuca rubra, Lathyurus pratensis*, *Gallium mollugo* and *Veronica chamaedrys* monocultures weighed less than 1g on average, whereas those in *Cynosurus cristatus*, *Bellis perennis*, and *Geranium pratense* monocultures weighed over 35g on average. The richness of plant functional groups had no influence on any of the measured variables of the transplants ($P > 0.05$). However, transplant biomass, height, LMA and probability to bolt or flower varied strongly between different species compositions within species richness levels.

The presence of small or tall herbs had no effect on aboveground biomass and plant height of the *K. arvensis* transplants whereas the presence of legumes had a positive

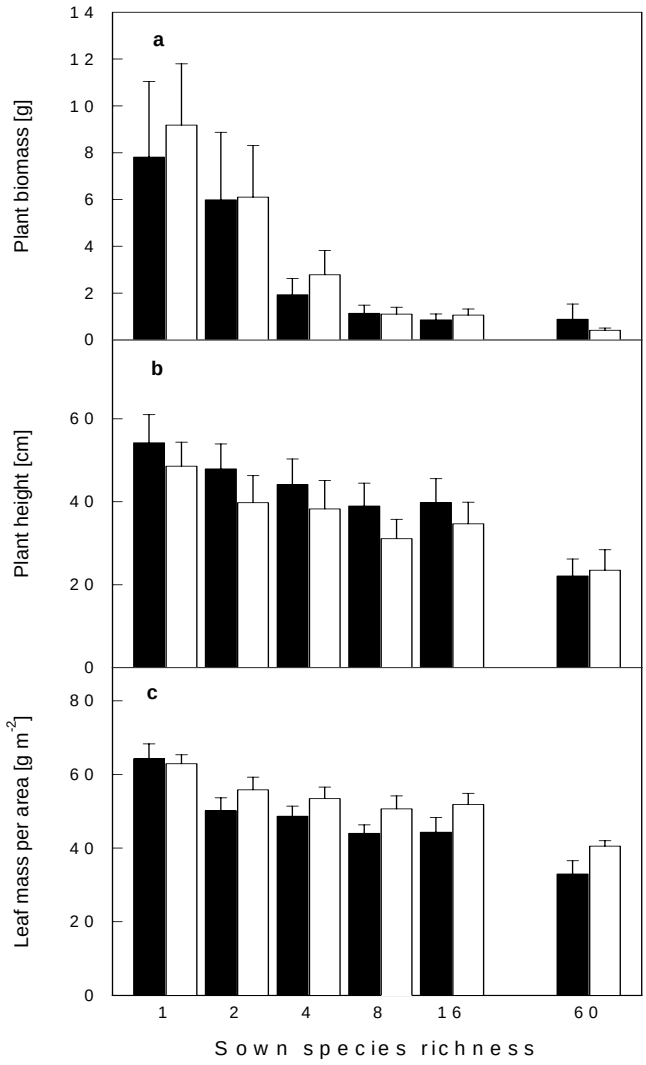

Figure 4.1 Effect of plant species richness on a) aboveground plant biomass, b) plant height, and c) leaf mass per area of ***K. arvensis*** transplants with aboveground competition (filled bars) and without (open bars) aboveground competition. Means + 1 s.e. are shown.

effect on aboveground biomass ($F_{1, 68}$ = 8.66, P = 0.004) and height ($F_{1, 69}$ = 9.93, P = 0.002) of the transplants. The presence of grasses had significant negative effects on both biomass ($F_{1, 68}$ = 9.90, P = 0.002) and height ($F_{1, 69}$ = 7.25, P = 0.009) of transplants (Fig. 4.3). On average, transplants in plots with grasses weighed only 1.7 g compared to 6.0 g in plots without grasses. The LMA of transplants was not affected by the presence of grasses, tall herbs or legumes (P > 0.05), but transplants in plots with small herbs had significantly lower LMA than transplants in plots without small herbs ($F_{1, 68}$ = 4.38, P = 0.040).

Table 4.1 Summary of analyses of variance using a general linear model for aboveground plant biomass, leaf mass per area, leaf nitrogen concentration and plant height of *K. arvensis* transplants. Significant values ($P < 0.05$) are in bold. [§]Residual d.f. for leaf mass per area, leaf nitrogen concentration and height are 70, 65 and 217 respectively because of some missing values and height data was per plant.

		Plant biomass			Leaf Mass per Area			Leaf Nitrogen Concentration			Plant Height		
Source of variation	**d.f.**	**MS**	**F**	***p*-value**	**MS**	**F**	***p*-value**	**MS**	**F**	***p*-value**	**MS**	**F**	***p*-value**
Between plots													
Block	3	9.72	3.692	**0.016**	1287.22	6.820	**<.001**	0.5601	1.127	0.344	3426.3	2.234	0.092
Species richness (linear-SR; log)	1	64.35	24.429	**<.001**	5324.30	28.211	**<.001**	0.8423	1.694	0.197	12521.4	8.165	**0.006**
Deviation from linear-SR	4	1.59	0.602	0.663	267.82	1.419	0.237	0.3779	0.760	0.555	560.6	0.366	0.832
Functional richness (linear-FG)	1	0.12	0.044	0.834	19.49	0.103	0.749	0.2207	0.444	0.507	52.0	0.034	0.854
Deviation from linear-FG	2	0.41	0.156	0.855	222.89	1.181	0.313	0.0249	0.050	0.951	151.1	0.099	0.906
Composition	69	2.63	4.540	**<.001**	188.73	2.830	**<.001**	0.4971	1.230	0.197	1533.5	3.671	**<.001**
Within plots													
Exclusion	1	1.08	1.850	0.178	852.85	12.81	**<.001**	0.2124	0.53	0.470	3084.0	7.383	**0.008**
Exclusion x linear-SR	1	0.02	0.030	0.865	239.13	3.59	0.062	0.0842	0.21	0.649	449.6	1.076	0.303
Exclusion x Dev-from linear-SR	4	0.29	0.490	0.740	64.91	0.98	0.427	0.0804	0.20	0.938	28.2	0.068	0.991
Residual	72	0.58			66.57			0.4030			365.5		

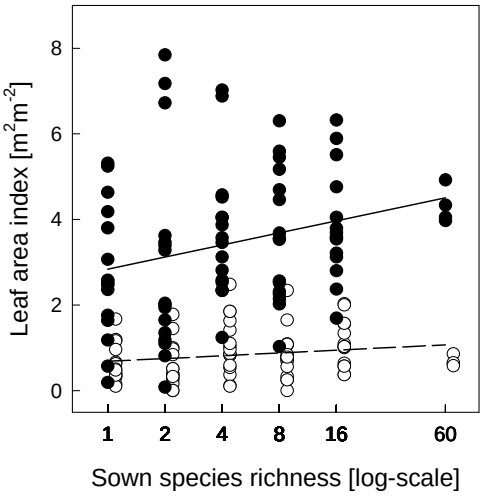

Figure 4.2 Interaction between plant species richness and removal of aboveground competition on leaf area index measured next to *K. arvensis* transplants. Closed symbols and solid line indicate LAI with aboveground competition; open symbols and broken line indicate LAI without aboveground competition. Note the symbols are slightly staggered along the x-axis for clarity.

Increasing species richness also reduced the probability of the transplants to bolt (produce stems) and to flower (Table 4.2 and Figure 4.4), and as expected, the mean number of flowers was strongly correlated with plant biomass ($r^2 = 0.76$, $P < 0.001$). The probability of the transplants to bolt was also low in plots with grasses (deviance ratio$_{1, 68}$ = 6.51, P = 0.013) and high in plots with legumes (deviance ratio$_{1, 68}$ = 3.93, P = 0.05). Concentration of nitrogen in the leaves of transplants was significantly lower in communities with grasses (1.86% compared with 2.23% N in all other communities, $F_{1, 68}$ = 12.169, P = 0.001) or small herbs (1.87% compared with 2.18% N, $F_{1, 68}$ = 4.56, P = 0.001) and increased in plots with legumes (2.30% compared with 1.83% N, $F_{1, 68}$ = 43.20, $P < 0.001$).

Effects of removal of aboveground competition

The LAI measured in the host community between the unmanipulated two transplants (i.e. with aboveground competition) increased with species richness (Fig. 4.2; $F_{1,73}$ = 5.21, P = 0.025). Furthermore, it was higher in communities with than without legumes ($F_{1,69}$ = 16.14, $P < 0.001$), whereas presence of grasses or herbaceous species had no significant effect on the

LAI. In contrast, the LAI of the host communities between the two transplants were aboveground competition was reduced, was markedly reduced ($F_{1,73} = 283.42$, $P < 0.001$) and this reduction was stronger in species-rich communities (Fig. 4.2; flat slope of species richness against LAI where aboveground competition was removed: $F_{1, 70} = 3.86$, $P = 0.053$). This shows that the exclusion treatment effectively reduced aboveground light competition.

Table 4.2 Analysis of deviance of probability of *K. arvensis* transplants to bolt and to flower. The mean deviance (m.dev.), the deviance ratios for the quasi-F test and the level of significance are shown, where *** $p < 0.001$, * $p < 0.05$ and $^{\sigma}$ $p < 0.1$. Residual d.f. for probability to flower is 217 because probability to flower was assessed at individual level.

		Probability to bolt			Probability to flower		
Source	**d.f.**	**m. dev**	**dev. Ratios**	**P**	**m. dev.**	**dev. ratios**	**P**
Between plots							
Block	3	1.999	1.018		2.661	**2.81**	*
Species richness (linear-SR; log)	1	7.472	**3.802**	$^{\sigma}$	20.460	**21.63**	***
Deviation from linear-SR	4	1.260	0.641		0.700	0.74	
Functional richness (linear-FG)	1	0.055	0.028		0.057	0.06	
Deviation from linear-FG	2	0.933	0.475		0.704	0.74	
Composition	70	1.965	**1.970**	***	2.471	**2.61**	***
Within plots							
Exclusion	1	0.401	0.400		0.198	0.21	
Exclusion x linear-SR	1	2.381	2.380		1.223	1.29	
Exclusion x Dev-from linear-SR	4	1.554	1.550		1.232	1.30	
Residual	70				0.946		

Despite this reduction of aboveground competition, the biomass of the transplants still declined with increasing species richness (Fig. 4.2a, non-significant interaction between the exclusion treatment and species richness in Table 4.1). Exclusion of the shoots of the neighbours also did not affect the probability of the transplants to bolt and to flower (Table 4.2) or their leaf nitrogen concentration (Table 4.1). There was a slight but non-significant indication that transplants in plots without grasses had slightly more

aboveground biomass when aboveground competition was reduced, whereas transplants in plots with grasses did not show such a response (Fig. 3).

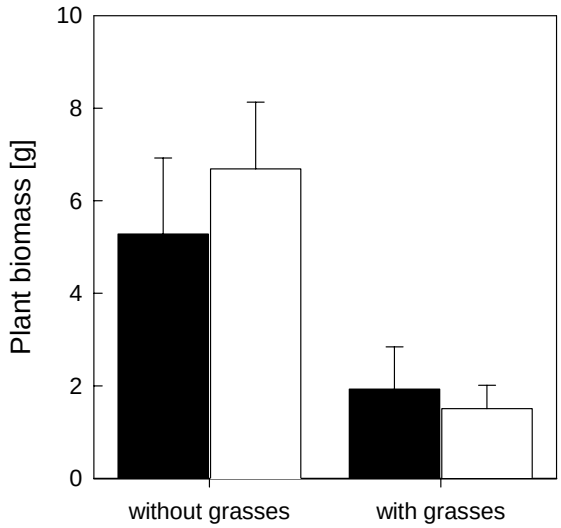

Figure 4.3 Aboveground plant biomass (mean + 1 s.e.) of *K. arvensis* transplants in communities with and without grasses, with aboveground competition (filled bars) and without (open bars) aboveground competition.

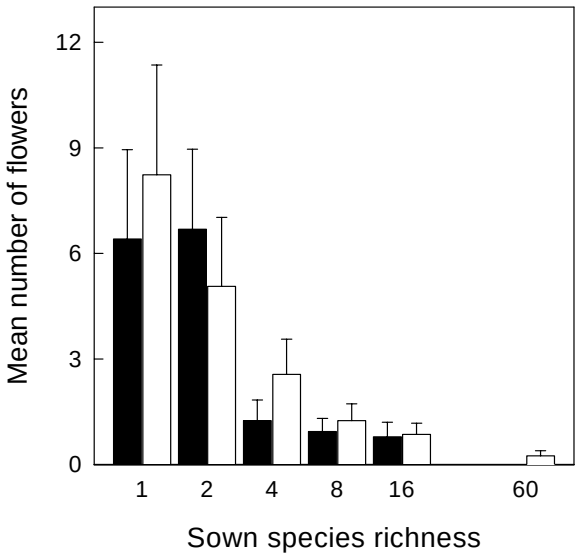

Figure 4.4 Effect of species richness on mean number of flowers in *K. arvensis* transplants (mean + 1 s.e.) with aboveground competition (filled bars) and without (open bars) aboveground competition.

Removal of aboveground competition revealed a typical morphological response to increased light availability; transplants were significantly shorter and had higher LMA than in the more shaded control treatment (Fig. 4.1b–c, Table 4.1). Nevertheless, there was no significant interaction between the removal of aboveground competition and species richness on these morphological variables.

To test if the effect of plant diversity on *K. arvensis* transplants was related to a changed light environment, i.e. aboveground resource use, we added the LAI as a covariate to our statistical model. However, LAI had no effect on transplant biomass ($F_{1, 69} = 2.10$, $P = 0.15$) and the species richness effect remained significant. The effect of LAI on the transplant LMA was significant ($F_{1,69}=21.7$, $P < 0.001$) thereby explaining part of the effects due to species richness and all the effect of removal of aboveground competition. LAI had no effect on the height of transplants, which was still significantly reduced by removal of aboveground competition, suggesting that this response was likely due to light quality (red/far red ratios) rather than light quantity. Leaf nitrogen concentration decreased with increasing LAI ($F_{1,70} = 11.67$, $P < 0.001$; Fig. 4.5) as expected from theory (Egli & Schmid 2000). However, the effect of removal of aboveground competition remained significant ($F_{1, 151} = 4.36$, $P = 0.041$), indicating that leaf nitrogen concentration of transplants were also influenced by other factors related to shoot competition, besides LAI.

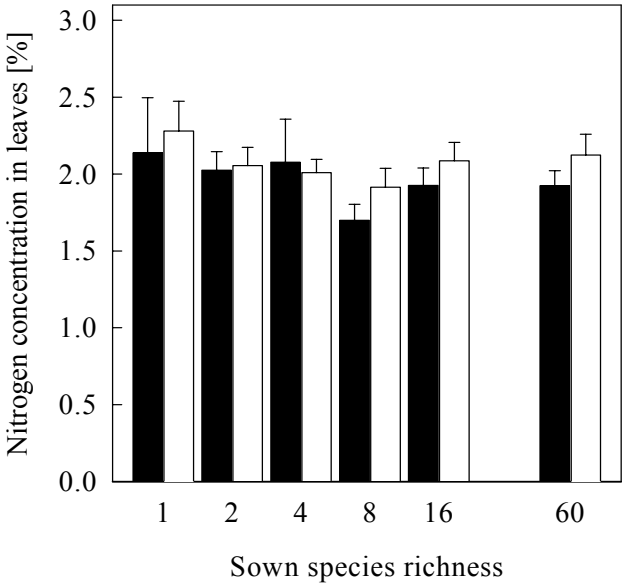

Figure 4.5 Effect of plant species richness on nitrogen concentration in the leaves of *K. arvensis* transplants with (filled bars) and without (open bars) aboveground competition.

Discussion

We investigated the performance of a plant species inserted into a fully established community from which it has been absent before. Our test species, *Knautia arvensis*, is not an exotic, but native species at our site, so that the term "invader" is here used in its widest sense, referring to "species invasions" rather than "invasive species". On first principles and as a null model, it seems reasonable to assume that the same mechanisms may allow successful establishment in plant communities of individuals of (i.e. invasion) both native or exotic species (Scherer-Lorenzen *et al.* 2007). Furthermore, we believe that using native species as test-invaders is more appropriate to understand community invasion resistance, because it avoids possible confounding effects of general and potential specific invasion mechanisms which might occur with exotics representing a biased selection of all potential invaders (Hierro *et al.* 2005; Mwangi *et al.* submitted).

Caveats

In our manipulation, we assumed that the main effect of bending down the neighbours of the transplants was a reduction in aboveground competition rather than affecting other aboveground interactions among plants, or between plants and other organisms. For example, differential herbivory among transplants with and without aboveground competition was unlikely because herbivory on *K. arvensis* in the same communities was very low (Scherber et al unpubl.). In addition, it is unlikely there was a difference in shoot-root ratio among the bent and intact neighbours, which might have reduced the positive effects on transplants without aboveground neighbours. We think that such effects are unlikely, because the bent-down neighbours were still green and growing during the full course of the experiment. Furthermore, a reduced investment in aboveground parts would reduce carbon uptake and eventually lead to reduced belowground biomass.

Effects of diversity

Our results are in line with previous findings indicating that plant diversity can increase (e.g. Knops *et al.* 1999; Levine 2000; Naeem *et al.* 2000; Spehn *et al.* 2000; Symstad 2000; Hector *et al.* 2001; e.g. Kennedy *et al.* 2002; Prieur-Richard *et al.* 2002; van Ruijven *et al.* 2003) and presence of grasses further enhance community invasion resistance (Crawley *et al.* 1999). Presence of legumes, on the other hand, can promote the growth of individuals added to a community because they fix atmospheric nitrogen and can thereby facilitate nitrogen uptake of other plants (Palmer & Maurer 1997; Prieur-Richard *et al.* 2002; Mwangi *et al.* in press). Only if legumes themselves are added to a community already containing legumes there usually is a negative effect on the invaders

(Fargione *et al.* 2003; Turnbull *et al.* 2005; Mwangi *et al.* in press). In our experiment, the non-leguminous test invader *K. arvensis* benefited from the presence of legumes in the plant communities. Nevertheless, *Lathyrus pratensis* and *Trifolium repens* monocultures were particularly suppressive to this test invader. Its is not clear why these legumes have strong negative effect on the test invader but it might be because they have a prostrate growth form unlike most other legumes in our communities.

Interestingly, the negative effects of plant diversity on the biomass of our test invader could not be explained by a diversity-related increase in LAI. The transplants did react to increasing LAI by increased plant height and reduced LMA, which can be interpreted as a typical shade-response (Huber 1996), although their aboveground biomass was unaffected. This suggests that the effect of plant diversity on the transplants was not through light quantity but possibly though light quality and other factors. Three-dimensional space filling is often suggested as a driver of invasibility (Knops *et al.* 1999; Naeem *et al.* 2000; Prieur-Richard *et al.* 2002) in studies that focus mainly on the recruitment stage, using seed-addition experiments. It is conceivable that once a newly added individual is established in a plant community, aboveground plant diversity effects are not so strong anymore and competition shifts from above to belowground (Wilson 1988; Casper & Jackson 1997; Cahill 1999). To test if there were some effects of aboveground competition in our study system, we applied the shoot exclusion treatment whose results are discussed below.

Our results do not support the theoretical prediction that presence of functionally similar species enhances invasion resistance: the presence of tall herbs in a plant community did not have an additional negative effect on *Knautia* transplants. However, our results do agree with previous findings that grasses confer high competitive

suppression of invaders (e.g. Crawley *et al.* 1999; Prieur-Richard *et al.* 2002; Fargione & Tilman 2005). We tentatively attribute this negative effect of grasses to depletion of soil resources, because transplants in plots with grasses had significantly lower nitrogen concentration in their leaves and because the presence of grasses did not increase the LAI and thus might have little effect on aboveground light competition.

Effects of removal of aboveground competition

Increased aboveground competition in more diverse plant communities may be important to suppress incoming plant individuals at early stages of their life cycle. Germination and seedling stages, which are mainly assessed in invasion studies, are particularly vulnerable to shading and space limitation. Disturbance that reduces aboveground competition and opens up space for recruitment is a major cause of invasion (Hobbs & Huenneke 1992; Burke & Grime 1996; Levine & D'Antonio 1999). It has therefore been suggested, that increased aboveground competition may play the major role in biodiversity-related invasion resistance (e.g. Naeem *et al.* 2000; Hector *et al.* 2001). This hypothesis can be tested by reducing aboveground competition in a biodiversity experiment, which we have done here for the first time.

In contrast to the suggestions, our results show that reducing aboveground competition does not reduce negative biodiversity effects on established plants of the test invader *Knautia arvensis*. We attribute this to a potential shift from above- to belowground competition in "control test invaders" as they become established and efficiently compete for light. Although light is a highly pre-emptable resource, plants usually have a great potential to respond to reduced light intensity by physiological responses leading to morphological plasticity such as elongation of internodes and

petioles or reducing LMA, allowing them to partly escape or avoid negative effects of shade (e.g. Schmid & Bazzaz 1994; Huber 1996). This was obviously the case with the established transplants of *K. arvensis* in our experiment.

The persistence of negative effects of plant diversity on the performance of established *K. arvensis* transplants even after removal of aboveground competition suggests that belowground competition is an important driver of community invasion resistance. The most likely explanation for this type of invasion resistance is increased belowground resource pre-emption rather than recruitment limitation as in seed addition studies. Several competition studies with established plants found a predominance of root competition over shoot competition (Wilson 1988; Wilson & Tilman 1995; Cahill 1999, 2003) and total competition shifts from above to belowground with decreasing soil fertility (Wilson & Tilman 1995; Casper & Jackson 1997; Cahill 1999, 2002a). Following four consecutive seasons of mowing and removal of plant material without fertilization in our experimental plots, soil resources were depleted to a greater extent in communities that were more diverse (Oelmann, unpublished data). This and the cumulative effect of roots (not harvested in contrast to shoots) may have intensified the magnitude of belowground competition relative to aboveground competition. In addition, removal of aboveground competition did not reduce the strong negative effect on the performance of *K. arvensis* transplants of the presence of grasses in a community. This suggests that the strong negative effect of grasses is not from aboveground competition; rather neighbouring grass shoots seem to be slightly facilitative. Grasses have slender and mostly erect leaves with relatively lesser shading, so the strong effect of grasses might have been due to their extensive root network that makes them efficient resource users (Craine *et al.* 2002; Fargione *et al.* 2003).

Morphological plasticity and changes in allocation of resources (allometry) along an environmental gradient are common phenomena in plant competition (Tilman 1988). *K. arvensis* showed a typical morphological response to aboveground competition: tall plants with thin leaves. Low leaf:stem ratio in transplants with full competition indicates an allometric response; that is, more allocation to stems allowing plants to position leaves higher in the canopy, which then leads to increased plant height. As such, the transplants could cope with aboveground but not with belowground competition. This suggests that mere observation of increased aboveground space filling and light interception with increasing diversity does not necessarily lead to increased competitiveness as reported before in some diversity–invasion studies (e.g. Fargione, *et al.* 2003). Our results show that belowground competition, especially from grasses, can promote invasion resistance of species-rich communities.

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Chapter 5

Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment

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Abstract

The importance of facilitative processes due to the presence of nitrogen fixing legumes in temperate grasslands is a contentious issue in biodiversity experiments. Despite a multitude of studies of fertilization effects of legumes on associated non-fixers in agricultural systems, we know little about the dynamics in more diverse systems. We hypothesised that the identity of target plant species (phytometers) and the diversity of neighbouring plant species would affect the magnitude of such positive species interactions. We therefore sampled aboveground tissues of phytometers planted into all plots of a grassland biodiversity–ecosystem functioning experiment and analysed their N concentration, δ^{15}N values and biomass. The four phytometer species (*Festuca pratensis*, *Plantago lanceolata*, *Knautia arvensis* and *Trifolium pratensis*) each belonged to one of the four plant functional groups used in the experiment and allowed the assessment of effects of diversity on N dynamics in individual species. We found significantly lower δ^{15}N values and higher N concentrations and N content (amount of N per plant) in phytometer species growing with legumes, indicating a facilitative role of legumes in these grassland ecosystems. Our data suggest that the main driving force behind these facilitative interactions in plots containing legumes was reduced competition for soil nitrate ("nitrate sparing"), with apparent N transfer playing a secondary role. Interestingly, species richness (and to a lesser extent functional group number) significantly decreased δ^{15}N values, N concentrations and N content irrespective of any legume effect. Possible mechanisms behind this effect, such as increased N mineralisation and nitrate uptake in more diverse plots, now need further investigation. The magnitude of positive interactions depended on the identity of the phytometer species. Evidence for increased N uptake in communities containing

legumes was found in all three non-legume phytometer species, with a subsequent strong increase in biomass in the grass *Festuca pratensis* across all diversity levels, and a lesser biomass gain in *Plantago lanceolata* and *Knautia arvensis*. In contrast, the legume phytometer species *Trifolium pratense* was negatively affected when other legumes were present in their host communities across all diversity levels.

Keywords: facilitation, nitrogen, δ^{15}N value, stable isotopes, The Jena Experiment

Introduction

The ability to fix atmospheric nitrogen is a key trait of most legume species in grassland or agricultural systems (Mosier 2002) and there can be multiple benefits for non-fixing plants species growing near N_2-fixers. N_2-fixers often have high tissue concentrations of N, which is released into the soil when plant parts die back, decompose and are mineralised (Tomm et al. 1995). Alternatively, N can be transferred more directly via root exudation and mycorrhizal links between different plants (McKey 1994, Uselmann et al. 1999, Shen and Chu 2004, Govindarajulu et al. 2005, Paynel et al. 2001). Non-fixing plant species, growing either in the vicinity of the N_2-fixers or growing on the same site after N_2-fixers have grown there, can take up this N and benefit from it, the process representing a model case for facilitation between species (Connell and Slatyer 1977, Bertness and Callaway 1994). Furthermore, soil N not taken up by the legumes due to N_2-fixation (spared N) also contributes to facilitative interactions with non N_2-fixing neighbours. Since legume species influence N dynamics of temperate European grasslands, their potential for improving the quantity and quality of agricultural fodder has been extensively studied (Vandermeer 1989, Hatch and Murray 1994, Murray and Clements 1998, Paynel et al. 2001).

In experiments looking at the effect of plant species loss on ecosystem processes, legume species have also been found to play a key role in influencing ecosystem N dynamics (Mulder et al. 2002, Spehn et al. 2002, Scherer-Lorenzen et al. 2003, Lambers et al. 2004, Palmborg et al. 2005) and community productivity (e.g. Tilman et al. 1997, Hector et al. 1999, Hooper and Vitousek 1997, Spehn et al. 2005) of the grassland ecosystems involved. As potential underlying mechanisms for positive diversity effects on biomass production, niche complementarity and also facilitation due

to N_2-fixers have been suggested (Loreau and Hector 2001, Roscher et al. 2005, Spehn et al. 2005, but see van Ruijven and Berendse 2003 for an example of positive diversity effects without legumes). However, in many of these experiments, diverse species mixtures always included legume species, so it has been argued that a major part of the positive diversity–productivity relationship can be attributed to the presence or absence of N_2-fixing legumes (an example of the sampling effect, Huston et al. 2000). One of the issues making a clear separation between effects of species richness and legume presence on ecosystem processes difficult, is that as species richness increases so does the probability of including a legume species in the mixture. On the other hand, as species richness increases the relative proportion of legume species (in plots with legumes) tends to decrease.

A current focus of biodiversity–ecosystem functioning experiments, therefore, is on the mechanisms behind the positive biodiversity effects on ecosystem primary productivity and how they change with time. Previous results suggest that several processes may be working at the same time to produce these positive biodiversity effects (Hector et al 2002, Roscher et al. 2005): the processes of niche complementarity and facilitation and a process known as the sampling (or selection effect). With niche complementarity and facilitation, trait differences among different species lead to more complete resource uptake from the environment, such that higher plant productivity (at community level) can be achieved by more diverse communities (Hector 1998, Tilman et al. 1997, Tilman 1999, Roscher et al. 2005, Spehn et al. 2005). In contrast, sampling effects occur because of the increasing chance of including certain key species or functional groups (e.g. legumes) with increasing community diversity in experiments using the random sampling with replacement method (Huston 1997, Wardle 1999).

Although methods have been developed to separate the sampling effect from complementarity and facilitation effects (Loreau and Hector 2001, Fridley 2001, Fox 2005), it is much harder to assess the relative roles of these latter two. Investigating facilitative processes more directly should thus help to elucidate the relative role of N_2-fixers in the higher productivity found in more diverse systems and will enable a better separation of facilitative from other complementarity effects in biodiversity experiments.

Since the potential of legumes to fix nitrogen can differ considerably (Carlsson and Huss-Danell 2003), both among species and under different environmental conditions (Sanford et al. 1995), the benefit to a neighbouring non-fixing plant will depend both on the capacity of the legume to fix nitrogen, and on the capacity of the non-fixing plant to benefit and gain a fitness advantage from the extra N input from its neighbour. Conversely, legume species can also negatively affect the neighbours via competition for other resources such as water and light (Tilman et al. 1997, Fargione et al. 2003). Because in a more diverse community more other non-legume species will compete for the nitrogen fixed by legumes the facilitative effect on a particular target species may be lower than in a less diverse community. We also know very little about the relative magnitude of N transfer during facilitation to plant species of different functional groups and its consequence for community structure and species abundances. Although low-diversity agricultural mixtures have been well studied (Sanford et al. 1995, Paynel et al. 2001, Høgh-Jensen and Schoerring 2000) facilitative effects in more natural systems, over a whole gradient of plant diversity, have received a lot less attention. The design of biodiversity experiments differs quite considerably from field agricultural experiments in that species mixtures are chosen at random from a specific

species pool, and many different mixtures representing a long gradient in species and functional group richness are investigated (Schmid et al. 2002). It is therefore to be expected that these systems representing a higher diversity gradient (including many plots without legumes) may show different N dynamics than those found in agricultural studies, which usually focus on specific low diversity mixtures of agricultural interest. The main drivers behind facilitative N dynamics in more complex ecosystems still remain to be elucidated. For example, Thomas and Bowman (1998) found decreased abundance of graminoids (versus forbs) when growing near *Trifolium* in alpine tundra, whereas results from a more species-rich temperate ecosystem indicate that grasses benefit more than forbs from growing in legume associations (Sanford et al. 1995, Oelmann et al. submitted), at least at the plant community level.

To investigate the importance of facilitative processes related to N_2-fixation within plant communities of different species richness, we used a biodiversity–ecosystem functioning experiment that showed a positive diversity–productivity relationship (see Roscher et al. 2005) to test the following hypotheses:

1) Non-legume plants growing with legume neighbours will have lower $\delta^{15}N$ values and increased N concentrations and N contents compared to those growing without legume neighbours;
2) Apparent transfer of fixed N_2 from legumes to other species depends on the species richness of the plant neighbourhood;
3) Four plant species representing four functional groups will respond differently to legume neighbours in terms of extra N acquired; a grass species will benefit more from legume presence than other non-fixers, due to a larger and more

effective root system, whereas a legume species will react neutrally to the presence of other legumes;

4) Any extra acquired N will lead to growth and hence fitness stimulation in the species involved.

To address these hypotheses, we used a phytometer approach (Mwangi et al 2006) with four transplanted species representing the four different functional groups (grasses, small herbs, tall herbs, legumes) of a biodiversity experiment in Jena, Germany. Evidence for facilitation related to N_2-fixation was investigated using the natural abundance $\delta^{15}N$ technique (Shearer and Kohl 1988, Högberg 1997) over two consecutive growing seasons. Apparent transfer of N can be assessed because N_2 fixed by legumes carries an isotopic signature of $\delta^{15}N$ closer to the atmospheric $\delta^{15}N$ signature (zero ‰) than N taken up from the soil (often positive $\delta^{15}N$ values). Consequently, $\delta^{15}N$ values in neighbours of N_2-fixers that are closer to zero (compared to those growing without legume neighbours) indicate that some transfer has probably taken place. Since phytometers growing with legumes may also benefit from soil nitrogen unexploited by legumes, differences in plant nitrogen status (N concentration, N content) together with the $\delta^{15}N$ signal in plant tissues indicate changes in N dynamics of the communities involved. In addition, relating N concentrations to N content in plants, one can assess whether any extra N uptake by phytometers growing with legumes resulted in a fitness (biomass) advantage. This assessment of the fitness consequences of N transfer and extra available N in legume plots was not previously possible in biodiversity experiments, since particular plant species occurred only in some of the randomly assembled plant communities. Only a phytometer approach as

used in the present study enables the investigation of the dynamics of N uptake and transfer to target species across the full experimental species richness gradient in such experiments.

Materials and methods

Experimental design

Our study was carried out as part of a larger biodiversity–ecosystem functioning experiment ("The Jena Experiment" http://www.the-jena-experiment.de) in Jena, Germany (Roscher *et al.* 2004). The experiment was set up in May 2002 on the outskirts of the town of Jena (50°55' N, 11°35' E; 130 m above sea level). The site lies in the floodplain of the river Saale, and was planted with vegetables and wheat for 40 years prior to the experiment. The area around Jena has a mean annual air temperature of 9.3 °C and mean annual precipitation of 587 mm (Kluge and Müller-Westermeier 2000). Due to flooding dynamics, the soil texture ranges from sandy loam near the river to silty clay with increasing distance from the river. Because of this gradient in soil characteristics, the site was divided into four blocks perpendicular to the river and diversity treatments were equally distributed among blocks.

Experimental communities were established in 82 large plots of 20 x 20 m with a gradient of species richness (1, 2, 4, 8, 16, 60 species) and functional group richness (1, 2, 3, 4 groups). The species were taken from a pool of 60 species that are typical to Central European mesophilic Molinio-Arrhenatheretum meadows (Ellenberg, 1996). The species from the 60-species pool were categorised into four functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species) by multivariate analysis using ecological and morphological traits (see Roscher

et al. 2004 for details). The mixtures were created by random selection (with replacement) producing a near orthogonal design, with 16 replicates per species richness level (Table 5.1) except for the 16-species (14 replicates) and 60-species richness level (4 replicates). We define mixture here as both multi-species and monoculture plots, for purposes of ease of language. It is important to note that the design of The Jena Experiment varies species richness and number of functional groups in an almost full factorial way – with obvious limitations at low diversities (it is not possible to have more functional groups than species) and at very high diversities due to the limited number of species per functional group within the regional species pool. Thus, up to the 16-species level, there are communities with and without legumes. For more details on the design, field site and exact species mixtures see Roscher *et al.* (2004).

Table 5.1 The replication of plant diversity treatments (species richness and functional group number) within The Jena Experiment's main experiment large plots, showing the number of different mixtures per treatment (in bold print).

		Species richness						No. of plots
		1	2	4	8	16	60	
Functional groups	1	**16**	**8**	**4**	**4**	**2**		**34**
	2		**8**	**4**	**4**	**4**		**20**
	3			**4**	**4**	**4**		**12**
	4			**4**	**4**	**4**	**4**	**16**
No. of plots		16	16	16	16	14	4	82

This provided a gradient of both plant species and functional richness. All functional groups at a given functional group richness level are generally sown in the same proportion, e.g. 50:50% in 2 FG plots, 33:33:33 % in 3 FG plots. There are exceptions to this, however, e.g. in 8 species and 3 FG plots, where sown legume proportion varies from zero through 0.25 to 0.275 percent. The four phytometer species were planted into every single plot of the experiment after establishment of the mixtures, thus allowing the investigation of diversity treatment effects on individual species.

All plots were mown twice a year in early June and early September corresponding to the typical management regime for extensive meadows in the region. Plots were weeded regularly during the growing season to maintain the sown species compositions. Weeds were cut out at ground level to avoid disturbing the soil. Mowing and weeding were done on a per block basis such that time differences could be taken into account by the block effect in the analysis. Plots were not fertilized during the experimental period.

Assessing the effect of diversity on individual species using transplanted phytometers

Because biodiversity experiments manipulate species and functional group richness as well as species composition, each particular species will only occur in a subset of experimental treatment plots. It is therefore difficult to assess the effects of these diversity treatments on a particular species. For this reason, we selected four species representative of the four functional groups and transplanted them as "phytometers" into each experimental plot in the second year of the experiment (2003). We define "phytometer" here as plant individuals transplanted into every plot of an already established experiment (Clements and Goldsmith 1924, Mwangi et al. 2006). The four species, *Festuca pratensis* Huds. (grass), *Plantago lanceolata* L. (small herb), *Knautia arvensis* L. (tall herb) and *Trifolium pratense* L. (legume) are all perennial and were chosen as representatives of their functional groups because of their high relative abundance in the surrounding *Arrhenatherum* grasslands. Individuals of the four phytometer species were pre-grown in potting trays filled with soil from the experimental site in a greenhouse for one month and transplanted into each large 20 x 20 m plot of the experiment in mid April 2003 at the phenological stage of 4–7 leaves.

Five randomly selected plants of each species were planted in lines forming a square of four species at distances of 28 cm intervals in a 2 x 2 m subplot within each plot. In total, 1640 individuals were transplanted into the 82 plots.

Plant Sampling

In the last week of August 2003, before the second mowing, the phytometer transplants were harvested at 5 cm above the ground. Five individuals of each species from each plot were pooled and dried at 70^{o} C to constant weight to determine aboveground biomass (Mwangi et al. 2006). Transplants of *T. pratense* and *P. lanceolata* had reached the reproductive stage at this point. The dried plant material was ground with a ball mill prior to chemical analysis. In the following year (2004), we sampled ten fully open leaves without petioles of each transplanted species (two leaves per individual) in each plot before the first mowing. In *P. lanceolata*, which has a rosette growth form, we cut off leaves at about 3 cm from the base.

Stable isotope analysis and N content calculation

For determination of isotope ratios and N concentrations, 3.5 ± 0.2 mg of dried and finely ground plant material per species per plot were weighed into tin capsules for analysis. Samples were analysed using an elemental analyser (EA 1100 CE, Milan) coupled through continuous flow with an isotope-ratio mass spectrometer (IRMS Delta C prototype refurbished to comply with specifications for a Delta+, Finnigan MAT). Sample ratios of $^{15}N/^{14}N$ are reported relative to the international standard for atmospheric nitrogen (calibration with IAEA-N1 0.43 ‰ compared to atmospheric N_2). Sample $\delta^{15}N$ values were calculated using the standard delta notation as follows:

$$\delta^{15}N = (R_{sample} / R_{standard} - 1) \times 10^3,$$

where R represents the ratio of $^{15}N/^{14}N$ isotopes. N concentrations per species per plot (%N) were also provided by the EA-IRMS analysis. The amount of N in the aboveground tissues of five sampled plants per plot was calculated by multiplying the dry mass of plant material by the corresponding nitrogen concentrations.

Possible limitations of the δ15N natural abundance method for assessing N transfer

Although the $\delta^{15}N$ natural abundance method has been frequently used to quantify the amount of N derived from symbiotic fixation and the transfer of fixed N to non-fixing neighbours (e.g. Høgh-Jensen and Schjoerring 1994), we refrained from aiming at a quantification of these processes due to several limitations of the method (Handley and Scrimgeour 1997, Högberg 1997, Gehring and Vlek 2004). Even the interpretation of qualitative evidence of N transfer has some limitations and is based on several assumptions that we could not test empirically within this study. 1) Since non-fixing species acquire N from several sources, their $\delta^{15}N$ signal will not only depend on the $\delta^{15}N$ signal of the neighbouring legumes, but additionally on several other factors, such as the $\delta^{15}N$ signal of soil organic matter and the amount of N mineralised from this soil organic matter and fractionation during this process.

In this study, we accounted for the spatial heterogeneity of bulk soil isotopic signals by assessing the effect of experimental blocks on $\delta^{15}N$ signals prior to fitting legume and diversity effects in the ANOVA model. Furthermore, we assumed any potential fractionation across the study site to be the same. 2) Soil $\delta^{15}N$ usually shows vertical gradients within the soil profile due to fractionation against $\delta^{15}N$ during the

mineralization–plant uptake pathway (Högberg 1997), and differential input of legume leaf or root litter at different depths. Differences in rooting depth among several receiver plants may thus lead to N uptake from differently labelled soil. However, we expect these differences to be rather small, as the field site was ploughed for 40 years before the onset of the experiment.

3) Finally, the process of N transfer itself may result in an enrichment of ^{15}N due to fractionation processes, but this should not differ among plant species (Högberg 1997).

Therefore, in our experiment, the effect of legumes may be considered an integrative effect of several factors that are influenced by legumes to various degrees. However, combining $\delta^{15}N$ signals with N concentrations within plant tissues, as recommended by Högberg (1997), provides a good tool to assess facilitative processes between different diversity treatments.

Testing the relationship between leaf and whole-aboveground-plant $\delta^{15}N$

In a separate measuring campaign, both whole aboveground plants and leaves of the experimental plant communities (excluding the phytometer plants) were harvested and analysed for $\delta^{15}N$. These data were used to test for any differences between leaf and whole plant $\delta^{15}N$, since for logistical reasons the 2003 phytometers harvest consisted of whole plants, but in May 2004 only of leaves. A comparison of community $\delta^{15}N$ per plot with leaf $\delta^{15}N$ per plot found that the two parameters correlate very closely, with leaf $\delta^{15}N$ values generally being slightly higher compared to whole-aboveground-plant $\delta^{15}N$ ($\delta_{leaf} = 0.794\ \delta_{whole\ plt} + 0.421$; $r^2 = 0.917$, $p < 0.0001$, $n = 28$). We therefore used both $\delta^{15}N$ data sets, without any correction, in the overall analysis over two growing seasons.

Statistical Analysis

The Jena Experiment has a factorial design with different levels of species and functional group richness as factors. Nevertheless, the design is not entirely balanced because the two factors depend on each other (e.g. it is impossible to have a plot with two functional groups but only one species). The design allows one, however, to assess the influence of different factors on a response variable in an analysis of variance with sequential sum of squares (Schmid et al. 2002). The analysis done here forms a split-plot factorial experiment with phytometer species nested within the main factorial design of The Jena Experiment. In all analyses, we fitted block first to remove variability due to spatial differences and then tested for the effects of the diversity treatments. By altering the order in which the diversity treatments were fitted we assessed the explanatory power of species and functional group richness ignoring or eliminating each other (Schmid et al. 2002). The design also allowed us to test for the effects of the presence (or absence) or the relative abundance of legumes (or other functional groups) in an experimental community on the response variables. In summary, the statistical design of this experiment tried to reduce confounding effects of species richness and legume presence or abundance in the experimental communities as much as possible. To account for differences between the two harvest times, we used repeated-measures ANOVA (using Genstat 6th Edition, Release 6.2. 2002). Homoscedasticity and normality were evaluated by plotting residuals and using Levene's test. All data fulfilled the assumptions of normality and homogeneity of variance.

The full ANOVA model used to analyse δ^{15}N, N concentration and N contents is shown in Table 5.2. As facilitation due to presence of legumes was of particular interest in this study, we also carried out analyses with the presence or absence or the relative abundance of legumes in an experimental community fitted before species richness or functional group number, to test for the different effects of this particular functional group regardless of diversity treatments. To determine the relative abundance of legumes we calculated their biomass proportion in mixtures at harvest. The interaction terms 'species richness x legume presence' (SR x LG) (Table 5.2) and functional group number x legume presence (FG x LG) were also fitted into the model.

According to the hierarchical structure of our experiment, error terms were mixture (MI), mixtures x phytometer species interaction (MI x PhS), mixtures x year interaction (MI x YR) and mixtures x phytometer species x year interaction (residual). Finally, we decomposed the phytometer species terms into a contrast of each against the others to test the particular differences among the transplant species. Species and functional group richness were also separated into linear contrast and deviations from linear terms to assess the functional relation between diversity treatments and response.

Results

Evidence for N2-fixation

The legume species in the experimental mixtures had significantly lower δ^{15}N values and significantly higher N concentrations than non-legume herbs and grasses growing in mixtures without legumes (Fig. 5.1, non-phytometer species). This clear separation of both groups, together with the δ^{15}N signal of the legumes around 0 ‰, is a clear indication that all legumes were actively fixing nitrogen (Högberg 1997). Thus, we can

assume that non-fixing phytometers growing in mixtures with legumes could potentially benefit from this additional N supply. *Trifolium pratense*, the only legume phytometer species was clearly different from the other three, confirming that it was also actively fixing N_2 (Fig. 5.1, Phytometer species).

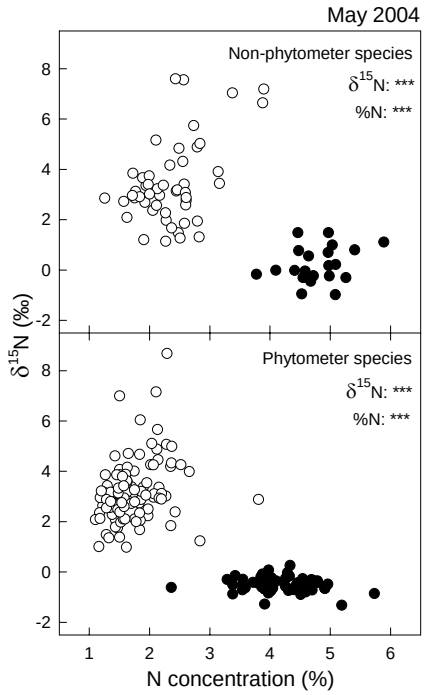

Figure 5.1 *Top panel:* The relationship between N concentration and $\delta^{15}N$ values in leaves of legumes (closed symbols) and non-legume species (open symbols) of all species sown in mixtures (i.e. non-phytometer species), in May 2004. Plants were sampled from mixtures with 1, 4 and 16 species only. *Lower panel:* The same relationship shown for the four phytometer species *Festuca pratensis*, *Plantago lanceolata*, *Knautia arvensis* (open symbols), and *Trifolium pratense* (closed symbols) in May 2004. Data of non-legume phytometers are from plots without neighbouring legumes only. ***: $P < 0.001$, two-tailed *t*-test.

Effects of legume presence and abundance

The first hypothesis that non-legume phytometer species growing with legume neighbours will have lower δ^{15}N values and higher tissue N concentrations and pools than those growing without legume neighbours was partly confirmed by our data. In an overall analysis of the data from both years (using repeated measures ANOVA; Table 5.2; Figs. 5.2 and 5.3) we found a clear positive effect of legume presence on N concentration in all phytometers. This effect was found irrespective of whether legume presence was fitted before ($F_{1,68}$ = 25.25, P < 0.0001) or after (Table 5.2) species richness. In contrast, legume presence had no significant effect on δ^{15}N values and N contents when fitted after species richness (Table 5.2), yet this effect was significant when fitted before species richness (δ^{15}N: $F_{1,68}$ = 10.01, P = 0.002; N content: $F_{1,68}$ = 4.40, P = 0.04). The effect of legume presence on N concentrations and N content in phytometer tissues was higher in May 2004 than in August 2003 (Table 5.2). N concentrations were 2–3 % in August 2003 compared to 2–5 % in May 2004, and maximum N contents in August 2003 were 100 mg per g of plant material, compared with 1200 mg per g in May 2004. In contrast, there was no significant difference in δ^{15}N values in phytometers between the two harvests.

The relative abundance of legumes in mixtures also had a significant effect on both N concentrations ($F_{1,68}$ = 61.95, P < 0.0001) and δ^{15}N values of phytometer species ($F_{1,68}$ = 7.42, P = 0.008) over the two years when fitted before the diversity treatments, although a significant relative abundance of legumes x year interaction indicated that this differed among years ($F_{1,70}$ = 7.62, P = 0.007 for N %; $F_{1,70}$ = 5.502, P = 0.022 for δ^{15}N). No significant interactions between species / functional group richness and relative abundance of legumes were found on either δ^{15}N values, N concentration or N contents (P = 0.642, P = 0.988, and P = 0.375, respectively).

Table 5.2 The effects of species richness, presence of legumes and time (year) on concentration of nitrogen in tissues, $\delta^{15}N$ ratios and N content in the phytometer species, using a repeated measures ANOVA (Type 1) for a split plot design (i.e. the phytometer species are nested within each plot). Degrees of freedom (d.f.), mean squares (M.S.), F ratios and *P*-values are provided for each fitted term in the hierarchical model; (*P*-values < 0.05 printed in bold face).

		N concentration (%)				$\delta^{15}N$ values				N content			
Source of variation	**Error term**	**d.f.**	**M.S.**	***F*-ratio**	***P*-value.**	**d.f.**	**M.S.**	***F*-ratio**	***P*-value**	**d.f.**	**M.S.**	***F*-ratio**	***P*-value**
Between Subject Factors													
Block	MI	3	0.528	1.037	0.382	3	9.912	3.672	**0.016**	3	149,083	1.635	0.189
Mono. vs. mixtures (MO)	MI	1	3.374	6.627	**0.012**	1	29.045	10.761	**0.002**	1	957,923	10.505	**0.002**
Spp. richn. (log-linear; SR)	MI	1	0.468	0.919	0.341	1	28.035	10.387	**0.002**	1	468,074	5.133	**0.026**
Legume presence (LG)	MI	1	18.226	35.801	**0.000**	1	7.281	2.698	0.105	1	40,723	0.447	0.506
MO x LG	MI	1	0.316	0.621	0.433	1	11.793	4.369	**0.040**	1	33,040	0.362	0.549
SR x LG	MI	1	0.750	1.474	0.229	1	0.004	0.001	0.971	1	36,146	0.396	0.531
Mixture (MI)	MI x PhS	73	0.509	2.646	**0.000**	73	2.699	4.853	**0.000**	73	91,184	2.076	**0.000**
Within Subject Factors													
Phytometer species (PhS)	MI x PhS	3	73.268	380.810	**0.000**	3	376.057	676.240	**0.000**	3	1,572,390	35.805	**0.000**
MO x PhS	MI x PhS	3	0.568	2.952	**0.034**	3	3.625	6.519	**0.000**	3	57,925	1.319	0.269
SR x PhS	MI x PhS	3	0.492	2.555	0.056	3	0.779	1.402	0.243	3	49,876	1.136	0.336
LG x PhS	MI x PhS	3	0.250	1.300	0.275	3	3.870	6.960	**0.000**	3	405,066	9.224	**0.000**
MO x LG x PhS	MI x PhS	3	0.073	0.381	0.767	3	3.566	6.412	**0.000**	3	96,152	2.190	0.090
SR x LG x PhS	MI x PhS	3	0.114	0.594	0.620	3	0.116	0.208	0.891	3	7,767	0.177	0.912
MI x PhS	Residual	214	0.192	1.428	**0.005**	214	0.556	1.389	**0.008**	213	43,915	0.828	0.915
Repeated Measures													
Year (YR)	MI x YR	1	17.457	76.130	**0.000**	1	1.593	1.929	0.169	1	5,036,954	79.182	**0.000**
MO x YR	MI x YR	1	0.433	1.888	0.174	1	1.455	1.761	0.188	1	350,647	5.512	**0.022**
SR x YR	MI x YR	1	0.193	0.840	0.362	1	0.107	0.130	0.720	1	336,628	5.292	**0.024**
LG x YR	MI x YR	1	0.375	1.633	0.205	1	0.546	0.661	0.419	1	39,624	0.623	0.432
MI x YR	Residual	75	0.229	1.702	**0.002**	75	0.826	2.063	**0.000**	75	63,612	1.200	0.163
PhS x YR	Residual	3	23.408	173.776	**0.000**	3	9.412	23.513	**0.000**	3	1,271,104	23.977	**0.000**
MO x PhS x YR	Residual	3	0.341	2.535	0.058	3	1.569	3.919	**0.010**	3	86,491	1.632	0.184
SR x PhS x YR	Residual	3	0.298	2.215	0.088	3	0.517	1.291	0.279	3	78,351	1.478	0.222

For 2004, we analyzed the role of the relative abundance of legumes on plant nitrogen patterns in those communities that contained legumes, because legume abundance is often mentioned as an important covariate that is confounded with effects of plant diversity in cases where the proportion of legumes increases with increasing diversity (e.g. Huston et al. 2000). Relative legume abundance had no effect on δ^{15}N values for either non-legume phytometers or *Trifolium*, (and also no effect on tissue N concentration and N content per plant for the latter; Fig. 5.4, Table 5.3). In contrast, both N concentration and N content substantially increased with relative legume abundance in non-fixing species (Fig. 5.4, Table 5.3).

Table 5.3 Linear regression analysis for dependence of δ^{15}N values, N concentration and N content (log) on legume proportion within those mixtures that contained legumes. Data from the harvest in May 2004. See figure 5.4 for data.

Species	δ^{15}N		N concentration (%)		N content (log) (mg plant^{-1})		Biomass (log) (g plant^{-1})	
	P	r^2	*P*	r^2	*P*	r^2	*P*	r^2
Festuca pratensis	0.185	0.06	**< 0.001**	0.39	**0.027**	0.17	**0.009**	0.16
Plantago lanceolata	0.598	< 0.01	**< 0.001**	0.54	**0.001**	0.25	**0.016**	0.13
Knautia arvensis	0.471	0.01	**0.002**	0.23	**0.047**	0.10	0.120	0.06
Trifolium pratense	0.413	0.02	0.101	0.07	0.123	0.06	0.102	0.07

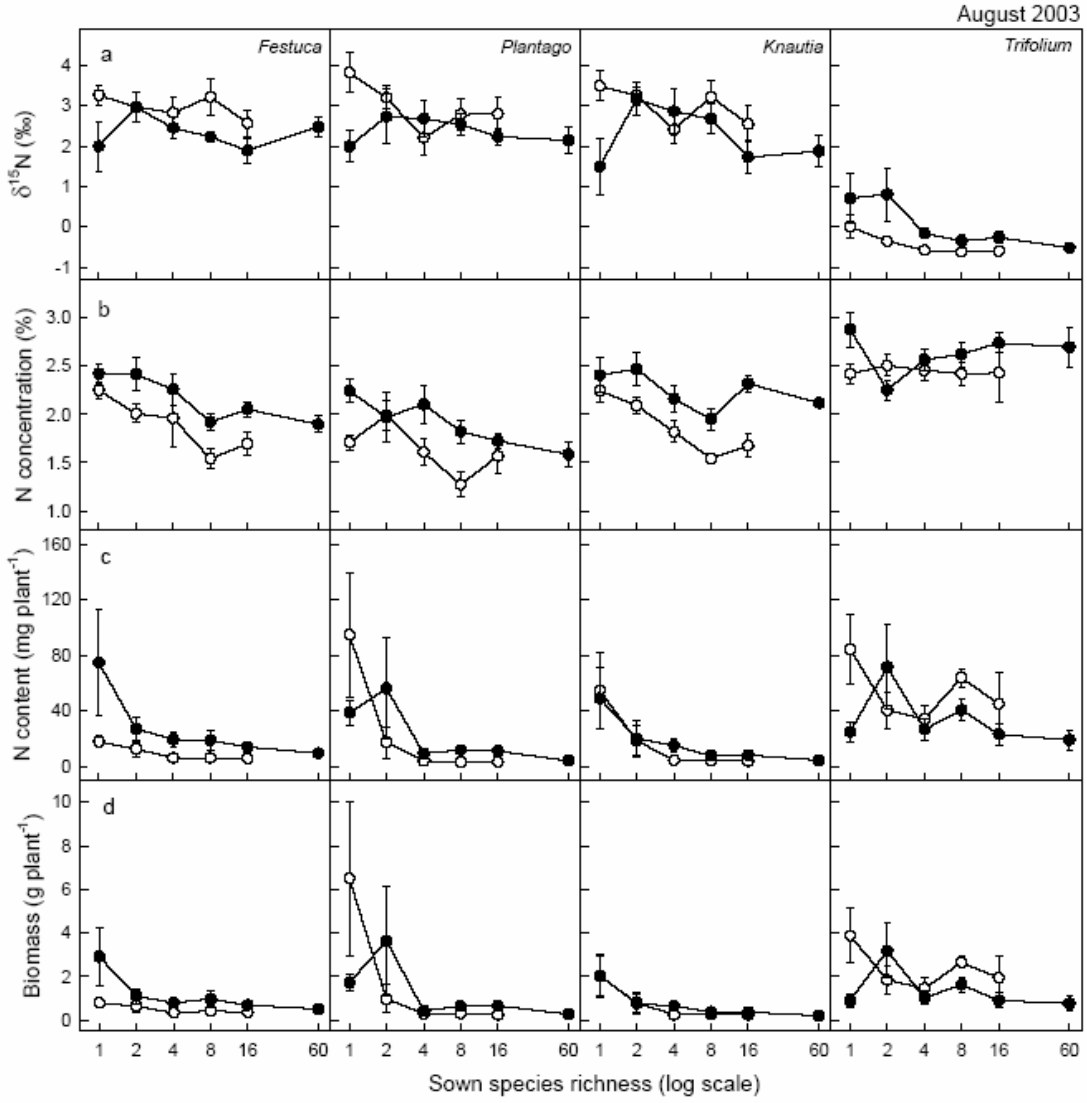

Figure 5.2 Effect of plant species richness and presence of legumes on a) δ^{15}N values, b) nitrogen concentration, c) N content, and d plant biomass in August 2003 for the four phytometer species. Biomass data are the aboveground biomass. *Open symbols* are plots without any legume species, *closed symbols* are plots with legume species present. Values are means with standard error.

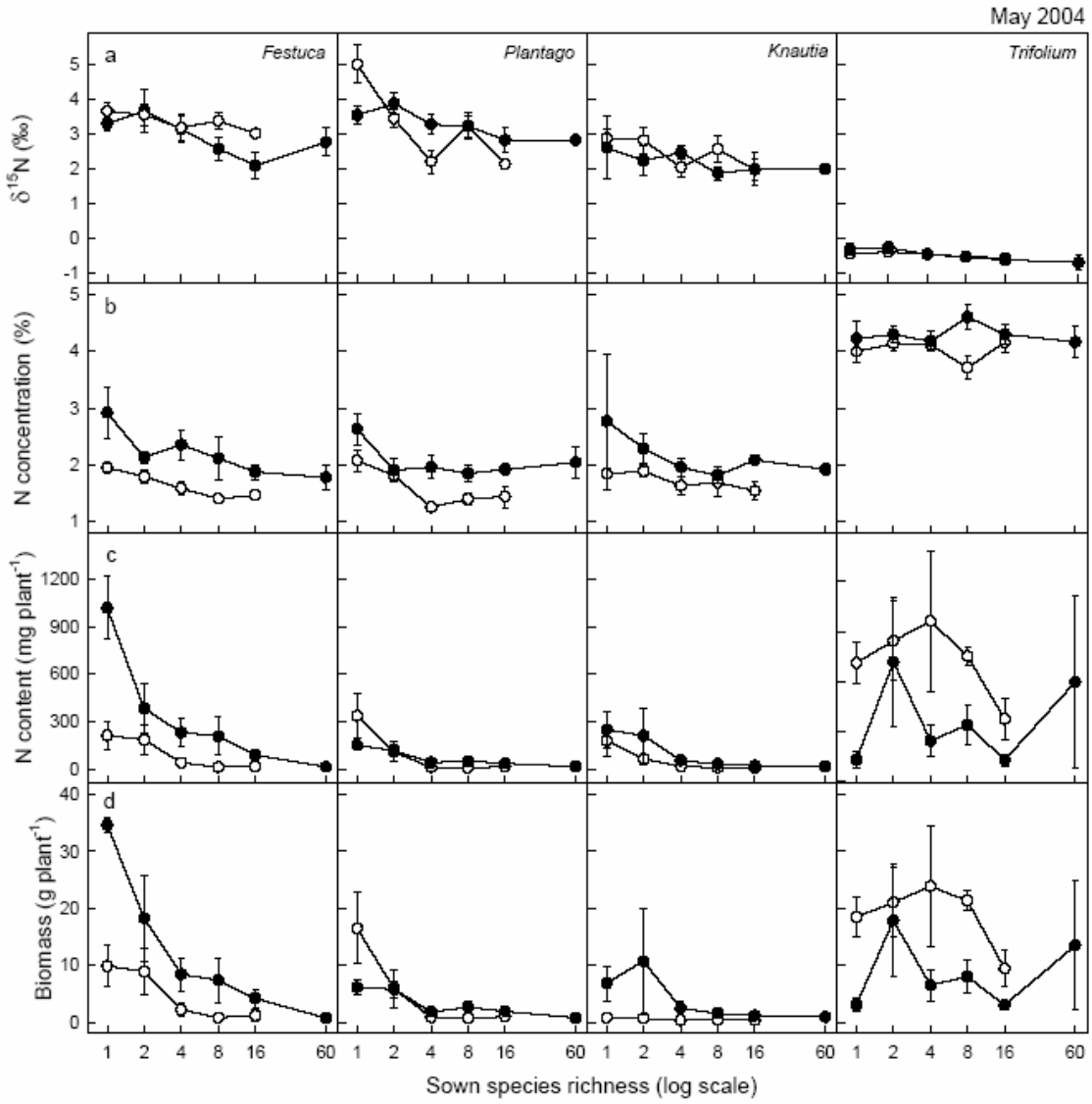

Figure 5.3 Effect of plant species richness and presence of legumes on a) δ^{15}N values, b) nitrogen concentration, c) N content, and d plant biomass in May 2004 for the four phytometer species. Biomass data are the aboveground biomass. *Open symbols* are plots without any legume species, *closed symbols* are plots with legume species present. Values are means with standard error.

Effects of plant diversity

Species richness had a significantly linear and negative effect on δ^{15}N values, N concentration, and N content in phytometer tissue; this was independent of legume presence for δ^{15}N values and N contents (see Figs. 5.2 and 5.3, and realised legume

abundance across the species richness treatments (Table 5.4). This diversity effect was due to a significant contrast between monocultures and mixtures for N concentration, whereas for δ^{15}N values and N contents both monoculture vs. mixture contrast and log-linear species richness contrast were significant (Table 5.2). The number of functional groups in the surrounding plant mixture had no effect, however, on either N concentration, N pools or δ^{15}N values of the phytometers after controlling for the species richness effect. When fitted *before* species richness, however, the number of functional groups had a significant effect on δ^{15}N and N content ($F_{3, 65}$, = 2.19, P = 0.004; $F_{3, 65}$ = 4.75, P = 0.005; respectively) but not on N concentrations. Species composition (mixture term) had a significant effect on N concentration, N content and δ^{15}N values in phytometer tissues. Interaction effects between species richness and phytometer species identity on δ^{15}N values and N concentration were due to differences between monocultures and mixtures (see monoculture *vs.* mixtures x phytometer species contrast: MO x PhS in Table 5.2).

Responses of different phytometer species

The four phytometer species clearly differed in N concentrations, N content and δ^{15}N values (Figs. 5.2, 5.3, 5.4 and Table 5.2). The four phytometer species also differed in their responses to legume presence with regard to tissue N concentrations and N contents (see significant interaction between legume presence and phytometer species for δ^{15}N values and N content in Table 5.2). At first sight, this is not surprising, since one of the phytometer species was a legume itself, and will *per se* have lower δ^{15}N values and higher N concentrations in its tissues than the other three species if it is fixing atmospheric N_2. When the difference between *Trifolium* and the other phytometer

species was removed (with a contrast *Trifolium* vs. other phytometers), however, significant differences between these other three were still found ($P < 0.001$ for subsequent contrast phytometer identity concerning the remaining three phytometer species). That is, subtle differences in apparent N transfer to different non-N_2-fixing phytometers also occurred: Figs. 5.2 and 5.3 show that *Plantago lanceolata* phytometers growing in two-species mixtures responded less strongly to legume presence than did the other two non-fixing species. Finally, contrasts between the grass phytometer species and the other two non-fixing phytometers were also all highly significant ($P < 0.001$).

Growth responses

Since we found evidence for increased N nutrition of non-legume phytometer species growing with legumes and that this was modulated by species richness (monocultures vs. mixtures), we assessed *a posteriori* whether this extra N was effectively invested into new growth (see Hypothesis 4 in Introduction). Figure 5.5 shows a "Timmer diagram" of N concentrations versus N content (Timmer and Morrow 1984) for each species richness level and split into the difference between legume and non-legume plots in May 2004. Clear evidence for growth stimulation due to legume neighbours (as indicated by crossing the isolines on the background, towards higher biomass values when moving from a non-legume to a legume neighbourhood) was found for *Festuca pratensis* and *Knautia arvensis*, except in monocultures for the latter species. *Plantago lanceolata* also showed some positive response in high-diversity communities, but it was suppressed in growth when growing in legume monocultures. Similarily, *Trifolium pratense* showed a strong reduction in growth at almost constant N concentrations (%) when growing in plots with other legumes present.

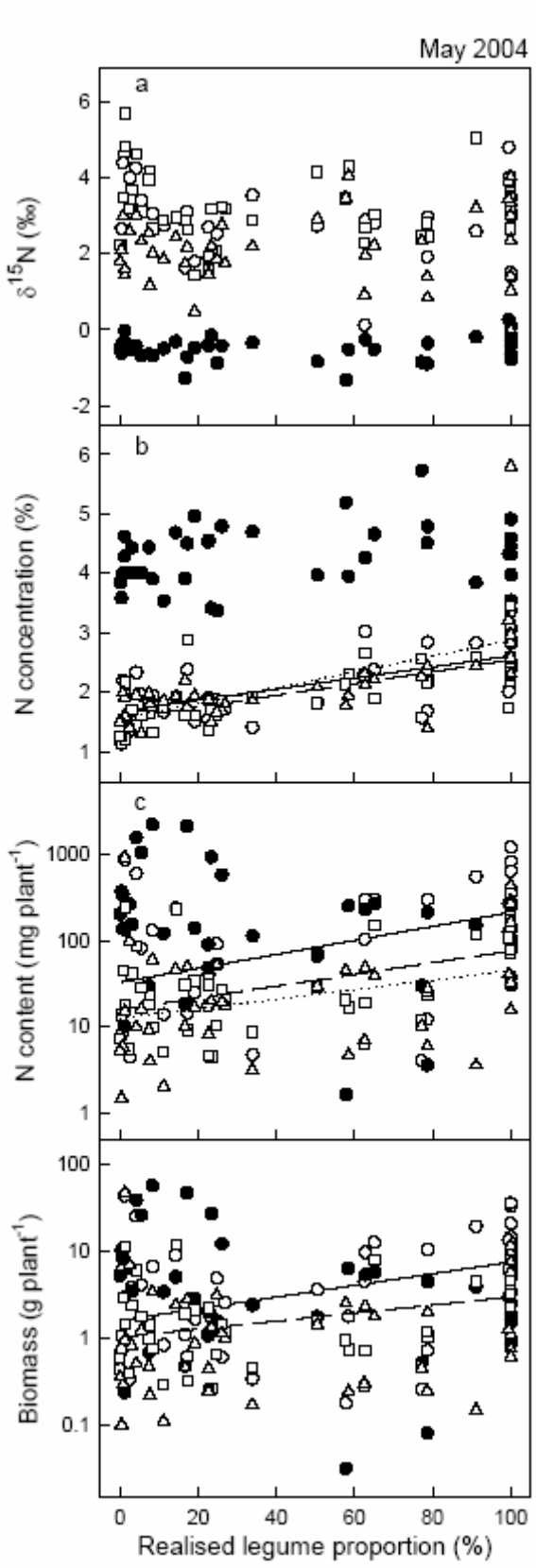

Figure 5.4 Influence of relative abundance of legumes on a) δ^{15}N values, b) nitrogen concentration, and c) N content (log-scale) of the phytometer species in mixtures containing legumes in May 2004. *Open symbols* are non-legume phytometers, *closed symbols* represent the legume phytometer. Lines for significant regressions are shown only (see Table 5.3 for statistics). *Circles* and *solid line*: *Festuca pratensis*, squares and dashed line: *Plantago lanceolata*, triangles and dotted line: *Knautia arvensis*.

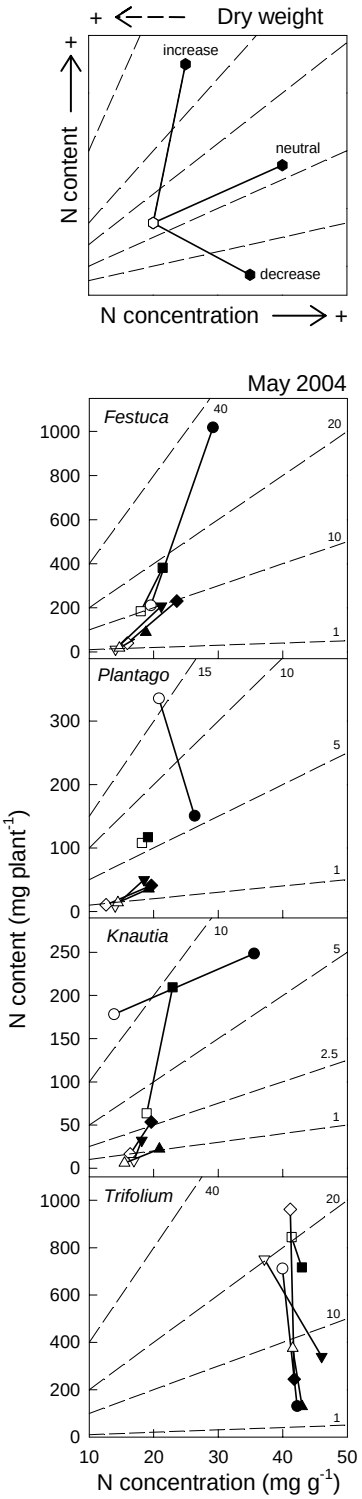

Figure 5.5 "Timmer diagrams" (Timmer and Morrow 1984) showing the change in the relationship between N concentration and N content when a phytometer species was growing with and without legumes neighbours in May 2004. Values are means (no standard error bars provided to simplify diagram) per species richness treatment, and the two mean values are linked by a straight line to help visualise the changes between mixtures without legumes (*open symbols*) and those with legumes (*closed symbols*). Biomass isolines (*dashed lines*, numbers attached indicate dry weight (in g) are presented as a visual aid so that one can read off the graph whether changes in N concentration and N content were accompanied by an increase, decrease or no change in biomass (i.e. fitness). *Top panel*: conceptual example of potential hypothesised changes. *Lower panels*: the relationship for the four phytometer species. *Filled circles*: monocultures, *filled squares*: two-species mixtures, *filled diamonds*: four-species mixtures, *filled inverted triangles*: eight-species mixtures, *filled triangles*: 16-species mixtures.

Table 5.4: Mean percentage of sown legume species per plot for each diversity treatment, in all plots and in just those plots containing legumes (bold).

		Species richness						No. of plots
		1	2	4	8	16	60	
Functional groups	1	25.0	25.0	25.0	25.0	0.0		34
		100.0	**100.0**	**100.0**	**100.0**			**8**
	2		25.0	25.0	25.0	25.0		20
			50.0	**50.0**	**50.0**	**50.0**		**10**
	3			25.0	25.0	25.0		12
				33.3	**33.3**	**33.3**		**9**
	4			25.0	25.0	25.0	20.0	16
				25.0	**25.0**	**25.0**	**20.0**	**16**
Average Sown		25.0	25.0	25.0	25.0	18.75	20.0	82
		100.0	**66.6**	**40.0**	**40.0**	**33.3**	**20.0**	**43**
Average Realized		25.8	19.1	24.2	23.4	29.6	27.4	
		100.0	**51.1**	**38.7**	**37.4**	**46.0**	**27.4**	

Values pertain to sown legume proportions except for the final line of the table, which relates to realized legume proportions over the two years of the study.

Discussion

Our hypotheses on the importance of facilitative effects between N_2-fixing legumes and neighbouring non-fixers rest on the assumption that nitrogen is a limiting resource for plant growth. Previous work in The Jena Experiment (Oelmann et al. submitted) established that N is a primary limiting nutrient in our meadow based on measurements of extractable mineral nitrogen. Additionally, in summer 2003 N:P ratios measured in aboveground tissues of plants were in the range of 5.9–9.5, well below the critical value of 16 for P limitation (Wassen et al. 2005). Finally, N-export by biomass harvests amounts to approximately 150 kg N ha^{-1} yr^{-1} (Scherer-Lorenzen, unpublished data) which is higher than mineral N production via mineralization or input through N-deposition. Given the fact that N is a limiting nutrient in The Jena Experiment, any

additional N input by symbiotic fixation may thus potentially lead to a substantial facilitative fertilization effect.

Effects of neighbour diversity on N dynamics

The results of our study focussing on four different phytometer species growing within a biodiversity experiment clearly indicate that legumes were fixing atmospheric N_2 and that non-legume phytometers benefited from increased N uptake when growing with legume neighbours. The main diversity effect was found both between monocultures and mixtures (for all N variables) but also amongst more diverse plots (for $\delta^{15}N$ and N content). It appears that, although functional group richness had some influence on $\delta^{15}N$ and N content in general, number of functional groups within a species richness level was not important. Conversely, species richness within a functional group (as well as in general terms) had a strong influence on $\delta^{15}N$ and N content. A similar result was obtained for other phytometer traits (Mwangi et al.2006). To help clarify the results from our relatively complex study, we first discuss differences between plots containing legumes or not (the legume effect), and then look at species richness effects within these two sets of plots.

Our results show that $\delta^{15}N$ values decreased, while tissue N concentrations and N contents within phytometer species increased when these species had legumes as neighbours, supporting earlier studies. In essence, the improved nutritional status of the phytometers may be due to several processes: (i) uptake of previously fixed N_2 through mineralization of legume tissue (ii) direct N transfer via interconnected mycorrhizal hyphae or rhizodeposition, or (iii) increased uptake of soil N that is not used by legumes because they rely more on atmospheric N_2 and are inefficient in capturing soil N (Chu et

al. 2004, Scherer-Lorenzen et al. 2003, Kahmen et al. 2006). Nevertheless, our data do suggest which of these processes may have played a main role and which a more supporting role in the facilitative interactions. Figure 5.4 shows that a strong effect of increasing realised legume proportion occurred in relation to plant N content and concentration but not in relation to δ^{15}N signal in plants. Had the apparent N transfer from legumes and non-legumes been the main mechanism of facilitation, we would have expected the δ^{15}N signal to be more significantly affected by legume presence and abundance than was actually the case. This leaves us with the alternative explanation that non-legume species benefited most from the so-called "sparing of N" (i.e. increased availability of soil N since the legumes relied more on atmospheric N sources than soil N). The lower δ^{15}N values in phytometers growing with legumes, however, suggest that the phytometers did receive some of their N from previously fixed atmospheric N_2. Data from agricultural studies show that the proportion of N transferred from legumes to receiver plants may add up to 40% of total N in mixed pastures (Ledgard and Giller 1995). In our study, however, it seems that N transfer played a subordinate role compared to N sparing, although a clear separation and quantification of these processes remains challenging.

We could show for the first time that δ^{15}N values, tissue N concentrations and N contents within individual plant species were negatively modulated by the diversity of the surrounding neighbourhood. This effect was found even when the legume effect was first removed from the data by using legume presence or abundance as a covariate. Table 5.4 shows that the proportional abundance of legumes did not in fact differ substantially across the species richness treatments. Even when assessing only those plots containing legumes, the proportional abundance of legumes realised (Table 5.4,

derived from harvest biomass) did not form a steep gradient which changed in unison with the species richness gradient. In addition, if the abundance of legumes realised did actually have confounding effects with species richness on the δ^{15}N signal, then one would have expected that decreasing legume abundance with increasing species richness should result in higher δ^{15}N signals. Our results show the opposite: in plots with and without legumes, the δ^{15}N signal in the phytometers was lower in the more diverse plots. A possible mechanism behind this overall effect could be that the many plant species (differing in their traits) in more species-rich systems are better able to take up the available soil resources (the so-called complementarity effect) (Mulder et al. 2002; Spehn et al. 2002; Palmborg et al. 2005). A consequence of this improved nutrient acquisition could be changes in N mineralisation rates (Palmborg et al. 2005) and improved carbon sequestration in the soil (S. Steinbeiss, personal communication).Changes in N mineralisation rates, in turn, may lead to changes in δ^{15}N signal in soil and plant N pools (Högberg 1997), with plant nitrate uptake fractionating slightly against the heavier isotope, producing lower δ^{15}N signals in plants. Such processes may account for the lower δ^{15}N signals found in phytometers growing in more diverse communities, irrespective of legume presence.

Given our current evidence for more positive interactions in the more diverse plant communities, it is perhaps surprising to see that the gain in N content or biomass was not as large as might be expected (Fig. 5.5) compared to biomass overyielding results found at the plant community scale (Roscher et al.2005). Our phytometer results relate to effects of diversity on single species and their populations, whereas reported complementarity or sampling effects on biomass or nutrient acquisition usually relate to the whole community level. Diversity seems to stabilize community and ecosystem

processes but not necessarily population processes (Tilman 1996). Further investigation of the mechanisms behind this species richness effect (irrespective of legume presence) on $\delta^{15}N$, N concentrations and N contents is now needed, taking possible complementarity and sampling effects at both the population and community levels into account.

Apparent N transfer affected by identity of the receiving species

We found that all three non-legume phytometer species benefited in terms of increased N uptake when growing in association with legumes across all diversity levels (with a few exceptions), but their response different considerably. We hypothesised that the grass species would benefit the most from N transfer from legume species, and this was indeed the case, and is consistent with observations made in low-diversity agricultural ecosystems (Sanford et al. 1995, Carlsson and Huss-Danell 2003, Xiao et al. 2004). A likely reason for indirect grass stimulation of N_2-fixation might be depletion of soil N by the grass, which would reduce nitrate inhibition by nitrate and nodule function, and increase the dependence of legumes on fixed N (Brophy et al. 1987, Sanford et al. 1995). Alternatively, the "nitrate sparing" found in soils with legumes (Chu et al. 2004, Scherer-Lorenzen et al. 2003) could be better exploited by the grasses with their extensive nitrophilic root system as highly efficient short-term soil N foragers. This more efficient exploitation of soil mineral N sources by grasses compared to herbs or legumes was recently reported by Kahmen et al. (2006) in managed temperate grasslands in Thuringia, Germany, using $\delta^{15}N$ labelling experiments. Our findings are also in agreement with evidence at the plant community level, where grass species in The Jena Experiment benefited more than herb species from growing in mixtures with

legume species, and where presence and abundance of legumes caused slightly higher, and presence of grasses slightly lower nitrate availability in the soil (Oelmann Y et al., Soil and plant nitrogen pools as related to plant diversity in an experimental grassland, submitted to Soil Sci Soc Am J). Spehn et al. (2002) found, in a similar biodiversity experiment, that the extent of N transfer to non-fixers varied depending both on the particular legume species and the particular non-legume species taking up the N. All four phytometer species are known to form symbioses with arbuscular mycorrhizae (Harley and Harley 1986) and probably do so in The Jena Experiment (Carsten Renker, personal communication), such that any apparent N transfer differences due to lack of mycorrhizal association can probably be ruled out.

Although we cannot infer the main mechanism behind the species-specific response to extra N uptake and apparent N transfer (whether due to differences in root exudation, mycorrhizal transfer or plant tissue decomposition or N mineralisation rates), we can assess whether the improved nutrient status of plants growing with legume neighbours actually produced a growth (fitness) advantage in the phytometers concerned. We define "fitness advantage" here as increased biomass production, since biomass is known to be positively correlated with the reproductive output of plants and is a measure of integrated plant performance. By plotting plant N concentration against N content on a background of biomass isolines (Fig. 5.5) for the May 2004 harvest, we assessed whether extra N transferred from legumes to phytometers caused a concomitant increase in biomass. Figure 5.5 shows the different fitness responses of a phytometer species to legume neighbours that are possible in theory. If the data line (in bold) moved along the biomass isoline, then fitness was maintained in the new situation. If the bold line moved from a lower biomass isoline to a higher one, then fitness

increased. The data from May 2004 indicate that all three non-legume phytometer species generally increased their fitness when growing with legumes, especially the grass *Festuca pratensis*. *Plantago lanceolata* phytometers growing in monocultures formed the exception, with a decrease in fitness in legume plots despite increased N concentrations. A possible explanation could be that *Plantago,* being a rosette species, suffered particularly from competitive shading effects of one (dominant) legume neighbour and was not able to derive a large compensatory advantage from extra N availability.

Not surprisingly, the legume phytometer *Trifolium* did not derive any fitness advantage from growing near other legume neighbours, in fact its fitness was reduced. In this species, an increase in N concentration did not lead to a fitness advantage. This is consistent with findings of a recent study by Turnbull et al. (2005), who found that experimental legume monocultures were more resistant to invasion by legumes than monocultures of non-legume herbs.

Conclusions

We provide clear evidence for facilitative interactions between legumes and non-legume neighbours in a biodiversity experiment in temperate grassland. In addition, we found that species richness generally decreased δ^{15}N values and N concentrations in phytometer species, and that this effect occurred irrespective of legume presence. This species richness effect was only partly due to a possible confounding factor of changing legume abundance with changes in species richness. Further investigation of this species richness effect on δ^{15}N values and N concentrations, which may be linked to changes in mineralisation rates, is now needed. Positive interactions in mixtures with legumes

seemed to be mainly linked to stimulated phytometer uptake of soil N not used by legumes (nitrate sparing) but also to a lesser extent to apparent N transfer from legumes. This suggests that both direct facilitative (N transfer) effects as well as other complementarity effects (improved resource use in more diverse systems with a variety of plant traits) played an important role in the plant communities investigated. A hierarchical partitioning of these underlying mechanisms could be one of the next steps in biodiversity-ecosystem functioning research. The magnitude of the positive response to legume neighbours differed between phytometer species, with the grass *Festuca pratensis* benefiting the most. All three non-legume phytometer species were able to gain a fitness advantage in mixtures with legumes, whereas the legume phytometer reacted negatively to legume neighbours. Thus, trait differences between species, e.g. related to belowground foraging for resources, are important for understanding positive interactions among legume and non-legume species and its effect on community dynamics and ecological processes.

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Chapter 6

Summary

Summary

Scientists believe we are already in the sixth extinction crisis, which unlike the past mass extinction episodes is driven by human activities. Conservative estimates by the IUCN[1] predict a loss of over four plant species per year worldwide. Other reports show that 22–47% of all plant species are threatened with extinction[2]. In addition to efforts in reducing human-induced rates of extinction, there is a great need to understand the possible consequences of this loss of biodiversity. This requires an integrated approach involving different disciplines, commensurate with the complexity of ecosystems.

The Jena Experiment in Jena Germany is one of such initiatives, which broadly seeks to determine the effect of changes in plant diversity on multi-trophic interactions, productivity, soil diversity, nutrients cycling, hydrology and plant population dynamics. This thesis is a product of the plant population dynamics sub-project of The Jena Experiment. On one hand, I sought to determine how local changes in plant diversity affect the remaining plant species. On the other hand, I wanted to determine how such changes influence the possibility of introduced species to establish. The second question also enabled me to evaluate how changes in plant diversity influence complementary and facilitative interactions between host community and introduced species.

In terms of community productivity, The Jena Experiment confirmed previous findings that loss of species reduces aboveground biomass. This suggests that loss of species may reduce carbon fixed from the atmosphere, thus less energy would enter into the system to support higher trophic levels. In *chapter 2* of this thesis, I show how this reduced primary productivity of the system is manifested in individual plants of a set of test species. I hypothesized that individual plants are bigger in species-rich communities

[1] The World Conservation Union (www.iucn.org)
[2] Pitman N.C.A., Jorgensen P.M., Williams R.S.R., Leon-Yanez S. & Valencia R. (2002) Extinction-rate estimates for a modern neotropical flora. *Conservation Biology*, 16, 1427-1431

because there are fewer neighbors of the same species, resulting in the observed increase in community productivity. However, despite the positive relationship between species richness and community productivity, the effect on individuals of the four test species that I evaluated varied greatly. Increasing species richness had positive effects on the biomass of individuals of *D. glomerata*, neutral effects on the biomass of individuals of *P. pratense* and *T. pratense* and negative effects on the biomass of individuals of *G. pratense*. This corresponded well with the effects of species richness on the population-level biomass in these species. Mortality of individuals of *D. glomerata* and *T. pratense* declined with increasing species richness leading to significantly increased numbers of individuals per seed sown in *D. glomerata*. This indicated that the positive effect of species richness on biomass of the entire population of *D. glomerata* was due both to increased numbers and sizes of individuals. The competitively superior *Arrhenatherum elatius* had a negative effect on the performance of individuals and population of the grass species, thus reducing the positive effects of diversity on these species. The results of this chapter show that individual species respond differentially to changes in species richness.

Understanding factors influencing invasibility of a plant community is a major goal in ecology. This is because invasion can negatively affect the integrity of an ecosystem. Using native species as test invaders to avoid confounding effects of invasiveness of exotic species, in *chapters 3* and *4* I report that plant diversity does indeed increase invasion resistance. Species richness enhances invasion resistance more strongly than does functional-group richness. In general, the presence of grasses increased invasion resistance of experimental communities, whereas herbs had neutral and legumes had positive effects. In addition, communities containing species of the same functional group as introduced species were most inhibitive to these test invaders, demonstrating that high niche overlap between native and introduced species enhances invasion resistance. To test if invasion

resistance was due to above- or belowground interactions between the host community and introduced species, in *chapter 4* I report results from an experiment in which I removed aboveground competition by neighbors around test invaders. I could thus demonstrate that belowground interactions were more important for invasion resistance than aboveground interactions: removing aboveground competition did not reduce the negative effects of species richness on the biomass of the test invader, *K. arvensis*. Furthermore, removal of aboveground competition revealed the typical shade-avoidance response of increased height growth and reduced leaf mass per area in the test species. In conclusion, root competition, especially by grasses, can render species-rich host communities highly suppressive of invaders, thus enhancing the invasion resistance of a host community.

It is well known that nitrogen-fixing legumes associated with other plants can increase yield in simplified agricultural systems. In *chapter 5*, we used the transplant approach to test if legumes have similarly beneficial effects in systems that are more diverse. We found that plants growing in communities with legumes had lower $\delta^{15}N$ values than communities without legumes, an indication that atmospheric nitrogen fixed by legumes "fertilized" the entire community. This was also reflected in higher nitrogen contents and concentrations in the transplanted "phytometer" individuals. However, species richness and to a lesser extent functional group richness of communities reduced the facilitative effects of legume presence. These negative effects of diversity on facilitation were partly due to decreasing legume abundance with increasing species richness. The transplanted phytometers of the four test species benefited differentially from the presence of legumes: increased N uptake was found in the three non-legume test species, with a subsequent increase in biomass only in the one grass test species; the legume test species *Trifolium pratense* was negatively affected by the presence of other legumes in the experimental communities.

In conclusion, the work in this thesis highlights the potential consequences of a further large-scale decline in plants diversity. Losses of plant species are increasing due to habitat change, habitat loss and habitat fragmentation, overexploitation, pollution, biological invasions and climatic change. These changes may affect individual species differentially but the overall effect is decline in energy entering into ecosystem (primary productivity). This can have knock-on effects on other ecosystem properties such as nutrient cycling, stability (e.g. drought and invasion resistance) and interactions among species, to mention a few. More studies are required at the level of individual plants or species in order to identify patterns that may enhance prediction of consequences of species loss on particular species. This is important for conservation, which mainly targets individual endangered species.

Zusammenfassung

Die wissenschaftliche Forschung sieht uns schon heute in einer sechsten Phase eines massenhaften Artensterbens, welches jedoch anders als in der Vergangenheit auf den Menschen zurückzuführen ist. Konservative Schätzungen der IUCN[3] gehen von einem Pflanzenartenverlust von vier Arten pro Jahr weltweit aus. Andere Untersuchungen zeigen, dass 22–47 % der Pflanzenarten vom Aussterben bedroht sind[4]. Ergänzend zu den Versuchen, das durch den Menschen verursachte Artensterben zu reduzieren, besteht eine grosse Aufgabe darin, die Folgen des Biodiversitätsverlusts zu erkennen. Dies erfordert einen integrierenden Ansatz, der durch die Einbindung unterschiedlicher Disziplinen der Komplexität eines Ökosystems Rechnung trägt.

Das *Jena Experiment* in Deutschland ist eine derartige Initiative, welche die Auswirkungen des Verlusts von Pflanzenarten auf verschiedene trophische Interaktionen, Produktivität, Diversität des Bodenlebens, Nährstoffkreislauf, Hydrologie und Populationsdynamik der Pflanzen untersucht. Die vorliegende Arbeit beschränkt sich auf das Teilprojekt innerhalb des *Jena Experimentes*, welches die populationsdynamischen Prozesse untersucht. Einerseits wollte ich feststellen, wie lokale Veränderungen der Diversität einer Pflanzengemeinschaft sich auf die verbleibenden Pflanzenarten auswirken. Andererseits versuchte ich abzuklären, wie solche Veränderungen die Etablierung eingewanderter Arten beeinflussen. Die zweite Frage ermöglicht darüber hinaus die Einschätzung der Bedeutung des Artenverlusts für Komplementarität und positive direkte oder indirekte Interaktionen in Pflanzengesellschaften.

Hinsichtlich der Produktivität einer Pflanzengesellschaft werden im *Jena Experiment* bisherige Forschungsergebnisse, die einen Artenverlusst mit einer geringeren

[3] The World Conservation Union (www.iucn.org)
[4] Pitman N. C. A., Jorgensen P.M., Williams R.S.R., Leon-Yanez S. & Valencia R. (2002) Extinction-rate estimates for a modern neotropical flora. *Conservation Biology*, 16, 1427-1431

Pflanzenbiomasse in Verbindung bringen, bestätigt. Daraus lässt sich folgern, dass mit dem Artenverlusst auch die Rate der Kohlenstofffixierung aus der Atmosphäre sinkt und somit weniger Energie für höhere trophische Ebenen zur Verfügung steht. Im Kapitel 2 stelle ich dar, wie sich diese geringere Pflanzenbiomasse im Hinblick auf das Individuum einer Pflanzengesellschaft manifestiert. Meiner Hypothese zufolge sind einzelne Pflanzen in artenreichen Gesellschaften grösser, da ihre Nachbarn seltener der gleichen Art angehören, und bedingen somit den Produktivitätszuwachs der Pflanzengemeinschaft. Im Gegensatz zu diesem positiven Zusammenhang zwischen Artenzahl und Produktivität einer Pflanzengemeinschaft, variieren die Auswirkungen der Artenzahl auf die Individuen von vier ausgewählten Arten sehr. Zunehmende Artenvielfalt hatte positive Auswirkungen auf die Biomasse der Individuen von *Dactylis glomerata*, keine Auswirkungen auf die Biomasse von *Phleum pratense*-Individuen, sowie negative Auswirkungen auf die Biomasseproduktion von *Trifolium pratense*-Individuen. Diese Ergebnisse stimmen gut mit den Auswirkungen der Artenzahl auf die Biomasseproduktion auf Populationsebene für dies Testarten überein. Die Sterberate von *D. glomerata*-Individuen und *T. prantense*-Individuen nahm mit zunehmender Artenzahl ab und führte zu einer hohen Dichte pro gesäte Samen von *D. glomerata.* Dies zeigte, dass der positive Effekt der Artenzahl auf die Biomasse der gesamten Population von *D. glomerata* sowohl auf die Erhöhung der Individualzahl, als auch auf die grösseren Individuen zurückzuführen war. Die konkurrenzstarke Art *Arrhenaterum elatius* hatte negative Auswirkungen auf die Leistung von Individuen und Populationen anderer Gräser und reduzierte damit den positiven Diversitätseffekt auf diese Arten. Die Ergebnisse in diesem Kapitel zeigen, dass die einzelnen Arten recht unterschiedlich auf Veränderungen der Artenzahl reagieren.

Das Verständnis der Faktoren, die die Invasionsfähigkeit einer Pflanzengesellschaft beeinflussen, ist ein wichtiges Ziel ökologischer Forschung, denn Invasionen fremder

Arten können negative Auswirkungen auf die Integrität eines Ökosystems haben. Die Verwendung von einheimischen Arten als Versuchspflanzen im Invasionsexperiment in Kapitel 3 und 4 verhindert konfundierende Effekte zwischen Invasion und dem Einfuss exotischer Arten. Dennoch lässt sich eine zunehmende Invasionsresistenz bei höherer Pflanzendiversität nachweisen. Dabei hat die Artenzahl einen stärkeren Effekt auf die Invasionsresistenz als die Anzahl funktioneller Gruppen. Im Allgemeinen verhindern Gräser das Eindringen fremder Arten in Pflanzegesellschaften. Demgegenüber wirken sich vorhandene Kräuter neutral und vorhandene Leguminosen positiv auf die Testinvasoren aus.

Pflanzengemeinschaften mit Arten, die der gleichen funktionellen Gruppe angehörten wie die fremde Testpflanze, konnten den Eindringling wirksamer unterdrücken. Darin kommt zum Ausdruck, dass sich überlappende ökologische Nischen der vorhandenen und der fremden Art die Invasionsresistenz erhöht. Um zu prüfen, ob die Invasionsresistenz auf ober- und unterirdische Pflanzeninteraktionen zurückzuführen sei, führte ich ein Experiment durch, in dem ich oberirdische Konkurrenz duch Nachbarn um die Testpflanzen herum entfernte (Kapitel 4). Die Resultate zeigen, dass unterirdische Interaktionen wahrscheinlich bedeutender sind als die oberirdischen: Ein Ausschalten oberirdischer Konkurrenz gegenüber der fremden Testpflanze *Knautia arvensis* reduzierte den negativen Einfluss der Artenzahl auf die Biomasse dieser Testpflanze nicht. Das oberirdische Entfernen der Nachbarn führte bei der Testpflanze zu einer bei Lichtkonkurrenz typischen morphologischen Reaktion: Die Pflanzen tolerieren eine oberirdische Konkurrenz mit vermehrtem Längenwachstum bei geringerem Blattgewicht, eine Strategie der Schattenmeidung, die in der Summe auch zu höherer Gesamtbiomasse führen kann. Meine Ergebnisse deuten darauf hin, dass die Wurzelkonkurrenz, vor allem bei Gräsern, in artenreichen Pflanzengemeinschaften ein hohes Potenzial zur

Unterdrückung invasiver Arten erzeugen kann und damit die Invasionsresistenz der gesamten Pflanzengemeinschaft fördert.

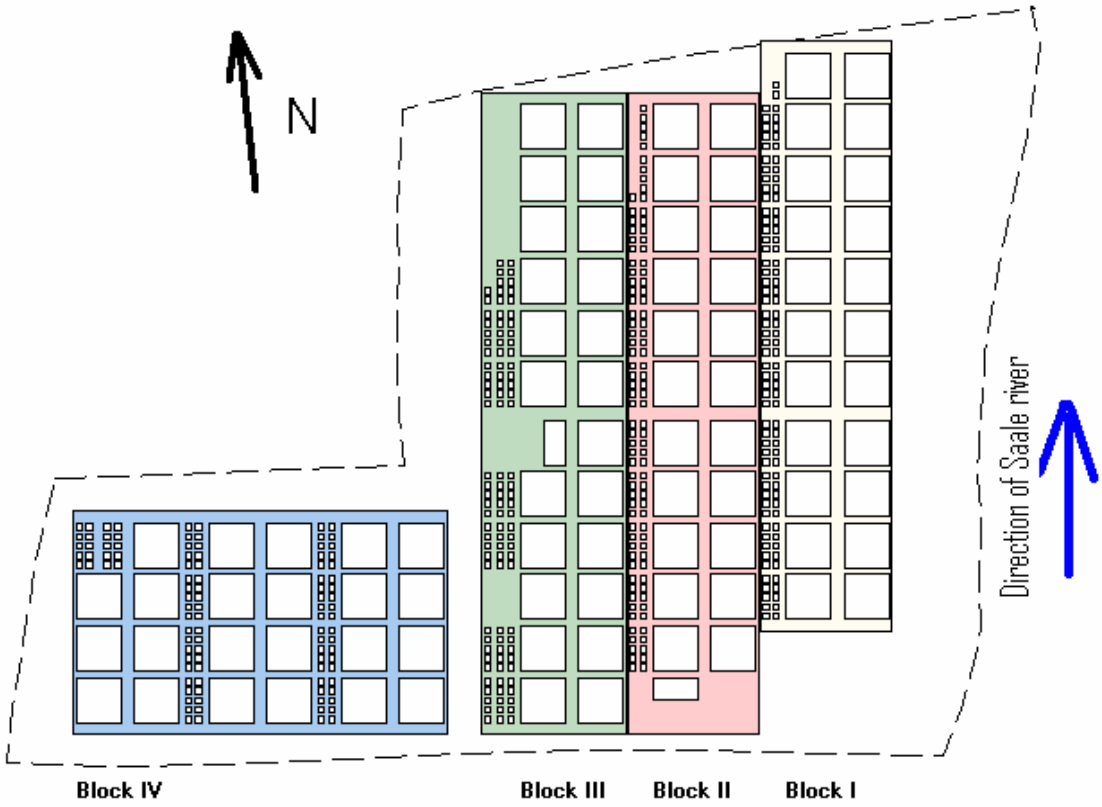
In artenarmen Landwirtschaftssystemen weist vieles darauf hin, dass Leguminosen sich positiv auf die nicht stickstofffixierende Begleitvegetation auswirken. In Kapitel 5 teste ich mit Hilfe von verpflanzten Testpflanzen („Phytometer“) die Hypothese, dass Artenvielfalt bei Pflanzen die fördernde Rolle der Leguminosen beeinflusst. Dabei zeigte sich, dass Pflanzen, die in Kombination mit Leguminosen wuchsen, niedrigere $\delta^{15}N$ Werte (ein Indikator für durch Leguminosen gebundenen Stickstoff) aufwiesen. Die höheren N-Konzentrationen und der höhere N-Gehalt pro Pflanze deuteten darauf hin, dass die Pflanzen tatsächlich von dem durch Leguminosen gebunden Stickstoff profitierten. Darüber hinaus hatte die Artenzahl (und in einem geringeren Masse die Anzahl der funktionellen Gruppen) einen negativen Einfluss auf die positive Wirkung der Legumiosen auf andere Pflanzenarten. Der negative Diversitätseffekt auf die positive Wirkung der Legumionsen hing zum Teil damit zusammen, dass die Anzahl der Leguminosen in den experimentellen Pflanzengemeinschaften mit steigender Artenzahl abnahm. Auch variierte dieser positive Effekt der Leguminosen zwischen den vier Phytometer-Arten. Hinweise auf eine erhöhte N-Aufnahme in Pflanzengemeinschaften mit Leguminosen konnten bei drei Testpflanzen, die nicht den Leguminosen angehörten, gefunden werden. Dies war verbunden mit einem Anstieg der Biomasse der Gras-Testarten. Demgegenüber wurde die den Leguminosen angehörige Testart *Trifolium pratense* negativ durch die Anwesenheit anderer Leguminosen in den Pflanzengemeinschaften beeinflusst.

Zusammenfassend hebt diese Doktorarbeit potentielle Konsequenzen einer weiteren grossflächigen Abnahme der Pflanzenvielfalt hervor. Verluste von Pflanzenarten infolge von Veränderung, Verlust und Fragmentierung des Lebensraumes, Übernutzung, Verschmutzung, biologischer Invasion und klimatischer Veränderung nehmen zu. Diese

Veränderungen können einzelne Arten unterschiedlich beeinflussen, aber der Gesamteffekt ist eine Abnahme der Energie, die in das Ökosystem einfliesst (Primärproduktion). Dies kann Dominoeffekte auf andere Ökosystemeigenschaften haben, so zum Beispiel den Nährstoffkreislauf, die Stabilität (z.B. Dürre- und Ivasionsresistenz) und die Interaktionen zwischen den Arten. Es braucht weitere Studien auf dem Niveau der individuellen Pflanze oder Art, um Muster identifizieren zu können, welche die Vorhersage der Konsequenzen des Artenverlusts auf bestimmte Arten verbessern. Dies ist auch wichtig für den Naturschutz, der hauptsächlich auf einzelne gefährdete Arten abzielt.

Appendices

Appendix 1 The site layout



Appendix 2 Species list categorised into four functional groups

Grasses	Small herbs	Tall herbs	Legumes
Alopecurus pratensis	*Ajuga reptans*	*Achillea millefolium*	*Lathyrus pratensis*
Anthoxanthum odoratum	*Bellis perennis*	*Anthriscus sylvestris*	*Lotus corniculatus*
Arrhenatherum elatius	*Glechoma hederacea*	*Campanula patula*	*Medicago lupulina*
Avenula pubescens	*Leontodon autumnalis*	*Cardamine pratensis*	*Medicago x varia*
Bromus erectus	*Leontodon hispidus*	*Carum carvi*	*Onobrychis viciifolia*
Bromus hordeaceus	*Plantago lanceolata*	*Centaurea jacea*	*Trifolium campestre*
Cynosurus cristatus	*Plantago media*	*Cirsium oleraceum*	*Trifolium dubium*
Dactylis glomerata	*Primula veris*	*Crepis biennis*	*Trifolium fragiferum*
Festuca pratensis	*Prunella vulgaris*	*Daucus carota*	*Trifolium hybridum*
Festuca rubra	*Ranunculus repens*	*Galium album*	*Trifolium pratense*
Holcus lanatus	*Taraxacum officinale*	*Geranium pratense*	*Trifolium repens*
Luzula campestris	*Veronica chamaedrys*	*Heracleum sphondylium*	*Vicia cracca*
Phleum pratense		*Knautia arvensis*	
Poa pratensis		*Leucanthemum vulgare*	
Poa trivialis		*Pastinaca sativa*	
Trisetum flavescens		*Pimpinella major*	
		Ranunculus acris	
		Rumex acetosa	
		Sanguisorba officinalis	
		Tragopogon pratensis	

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